bordeaux neurocampus

INTERNATIONAL BORDEAUX NEUROCAMPUS BRAIN CONFERENCE

bordeaux neurocampus BRAIN

Astrocytes & Microglia: Key Partners in Synaptic Transmission

BORDEAUX, SEPT. 30 - OCT. 2, 2015

INSTITUT D'OPTIQUE D'AQUITAINE (TALENCE)

Scientific Organizers

Elena Avignone
Agnès Nadjar
Aude Panatier

Invited Speakers

Etienne Audinat, FR
Alain Bessis, FR
Giorgio Carmignoto, IT
Carole Escartin, FR
Marc Freeman, US
Wenbiao Gan, US
Cornelius Gross, IT
Sophie Layé, FR
Giovanni Marsicano, FR
Stéphane Oliet, FR
Richard Ransohoff, US
Richard Robitaille, CA
Nathalie Rouach, FR
Dmitri Rusakov, UK
Mike Salter, CA
Beth Stevens, US
Marie-Eve Tremblay, CA

Travel grants for students available

brainconf.u-bordeaux.fr

cnrs Inserm Institut national de la santé et de la recherche médicale INRA SCIENCE & IMPACT université de BORDEAUX RÉGION AQUITAINE EXCELLENCE université de Bordeaux INITIATIVE INVESTISSEMENTS D'AVENIR

The second edition of the International Bordeaux Neurocampus / Brain Conferences is scheduled from September 30 to October 2, 2015. "GliSyn" for "Astrocytes and microglia, key partners in synaptic transmission", will focus on the possible partnership of two glial cells, astrocytes and microglia, in synaptic transmission. It will take place at the Institut d'Optique d'Aquitaine (a part of the Institut d'Optique Graduate School, IOGS) at Talence, southwest of the famous city of Bordeaux, on the University campus of Bordeaux.

Although astrocytes and microglia share common features, the astrocyte and microglia fields are growing independently. The goal of this conference is to gather for the first time scientists from both fields. Three sessions will be dedicated to the influence of the two types of glial cells at the synapse during development, in adult physiology and in pathology.

Renowned Speakers will be there to brainstorm with more than 200 researchers, coming from all over the world. The scientific program of this meeting is based on plenary lectures made by world-class personalities. Several young scientists will be selected on their abstracts by the Scientific Committee to give a talk during the event. Poster sessions will also be organized for informal exchanges among participants. Finally, to stimulate discussion about the possible partnership of astrocytes and microglia in synaptic transmission, round tables will be organized at the end of each session.

An exhibition area will host industrial stands providing an excellent opportunity to interact with companies in different domains and familiarize themselves with the latest technological advances.

The Invited Speakers are:

Etienne AUDINAT, Université Paris Descartes - FR
Alain BESSIS, IBENS Paris - FR
Giorgo CARMIGNOTO, Neuroscience Institute, National Research Council, Padova - IT
Carole ESCARTIN, MIRCen Fontenay-aux-Roses - FR
Marc FREEMAN, University of Massachusetts Medical School - US
Wenbiao GAN, Skirball Institute New-York - US
Cornelius GROSS, EMBL Monterotondo - IT
Sophie LAYE, NutriNeurO Bordeaux - FR
Giovanni MARSICANO, NCM Bordeaux - FR
Stéphane OLIET, NCM Bordeaux - FR
Richard RANSOHOFF, Lerner Research Institute Cleveland - US
Richard ROBITAILLE, Université de Montréal - CA
Nathalie ROUACH, Collège de France Paris - FR
Dmitri RUSAKOV, University College London - UK
Michael SALTER, University of Toronto - CA
Beth STEVENS, Children's Hospital of Boston - US
Marie-Eve TREMBLAY, University of Laval Québec - CA

Organizing Committee in Bordeaux:

Elena AVIGNONE, Maître de conférences - Institut interdisciplinaire de neurosciences, IINS
Agnès NADJAR, Maître de conférences - NutriNeurO
Aude PANATIER, Chargée de recherche CNRS - NeuroCentre Magendie, NCM

The Organizing Committee thanks the institutional and academic partners:

as well as the partners which supported the conference:

GLISYN AT A GLANCE

Wednesday, September 30th

08:15 - 09:15	**REGISTRATION**		
09:15-09:45	Welcome remarks by Daniel CHOQUET		
Session 1 -	**ROLE OF GLIAL CELLS DURING DEVELOPMENT** *Chaired by Agnès NADJAR and Giorgio CARMIGNOTO (a.m.)*		
09:45-10:15	Cornelius GROSS	–	IS 001
10:15-10:45	Sophie LAYE	–	IS 002
10:45-11:10	*Coffee break*		
11:10-11:30	Selected talk: Anne ROUMIER	–	ST1.1
11:30-12:00	Etienne AUDINAT	–	IS 003
12:00-13:00	*Lunch break*		
13:00-15:00	Poster session		
Session 1 -	*Chaired by Nathalie ROUACH and Cornelius GROSS (p.m.)*		
15:00-15:30	Beth STEVENS	–	IS 004
15:30-16:00	Marc FREEMAN	–	IS 005
16:00-16:30	*Coffee break*		
16:30-16:50	Selected talk: Joana FERREIRA	–	ST1.2
16:50-17:20	Richard ROBITAILLE	–	IS 006

Thursday, October 1st

Session 2 -	**ROLE OF GLIAL CELLS IN SYNAPTIC TRANSMISSION** *Chaired by Etienne AUDINAT and Richard ROBITAILLE (a.m.)*		
09:15-09:45	Wenbiao GAN	–	IS 007
09:45-10:15	Marie-Eve TREMBLAY	–	IS 008

10:15-10:45	Alain BESSIS	–	IS 009
10:45-11:10	*Coffee break*		
11:10-11:30	Selected talk: Thomas PFEIFFER	–	ST2.1
11:30-12:00	Stéphane OLIET	–	IS 010
12:00-13:00	*Lunch break*		
13:00-15:00	Poster session		

Session 2 - *Chaired by Aude PANATIER and Alain BESSIS (p.m.)*

15:00-15:30	Dmitri RUSAKOV	–	IS 011
15:30-16:00	Nathalie ROUACH	–	IS 012
16:00-16:30	*Coffee break*		
16:30-16:50	Selected talk: Jonathan ZAPATA	–	ST2.2
16:50-17:20	Giovanni MARSICANO	–	IS 013
17:20-18:30	Round table: Brainstorming on the role of microglia and astrocytes at the synapse		

19:00 -23:30 ***Gala dinner*** *at Château Carbonnieux*

Friday, October 2nd

Session 3 - **ROLE OF GLIAL CELLS IN BRAIN PATHOLOGY**
Chaired by Elena AVIGNONE and Stéphane OLIET (a.m.)

09:00-09:30	Richard RANSOHOFF	–	IS 014
09:30-10:00	Michael SALTER	–	IS 015
10:00-10:20	Selected talk: Li TIAN	–	ST3.1
10:20-10:40	*Coffee break*		
10:40-11:00	Selected talk: Eric MARTINEAU	–	ST3.2
11:00-11:30	Carole ESCARTIN	–	IS 016
11:30-12:00	Giorgio CARMIGNOTO	–	IS 017
12:00-12:30	Closing remarks by Christophe MULLE		
12:30	Departure: *Lunch box*		

GLISYN PROGRAMME

Wednesday, September 30th

08:15 - 09:15 **REGISTRATION**

09:15-09:45 Welcome Remarks by **Daniel CHOQUET**

Session 1 - **ROLE OF GLIAL CELLS DURING DEVELOPMENT**
Chaired by Agnès NADJAR and Giorgio CARMIGNOTO (a.m.)

09:45-10:15 **Cornelius GROSS**, EMBL Monterotondo (IT)
Microglia contribute to autism-related functional connectivity and behavioral deficits

10:15-10:45 **Sophie LAYE**, NutriNeurO Bordeaux (FR)
Lipid nutrition regulates microglia activity and function: consequence on behavior

10:45-11:10 *Coffee break*

11:10-11:30 Selected talk: **Anne ROUMIER**, Institut du Fer à moulin Paris (FR)
Modulation of microglia by serotonin 5-HT_{2B} receptors: possible implications for developmental synaptic refinement

11:30-12:00 **Etienne AUDINAT**, Université Paris Descartes Paris (FR)
Microglia and maturation of synaptic circuits during cortical development

12:00-13:00 *Lunch break*

13:00-15:00 Poster session

Session 1 - *Chaired by Nathalie ROUACH and Cornelius GROSS (p.m.)*

15:00-15:30 **Beth STEVENS**, Children's Hospital of Boston (US)
Immune mechanisms of synapse loss in health and disease

15:30-16:00 **Marc FREEMAN**, University of Massachusetts Medical School (US)
Roles for astrocytes in neural circuit assembly

16:00-16:30 *Coffee break*

16:30-16:50 Selected talk: **Joana FERREIRA**, IINS Bordeaux (FR)
Co-agonist tunes GluN2B-NMDA receptor trafficking and content at developing hippocampal synapses

16:50-17:20 **Richard ROBITAILLE**, Université de Montréal (CA)
Glial cells regulate synaptic properties to alter the outcome of synaptic competition at the mammalian neuromuscular junction

Thursday, October 1st

Session 2 - **ROLE OF GLIAL CELLS IN SYNAPTIC TRANSMISSION**
Chaired by Etienne AUDINAT and Richard ROBITAILLE (a.m.)

09:15-09:45 **Wenbiao GAN**, Skirball Institute New-York (US)
In vivo studies of microglial function in synapse formation

09:45-10:15 **Marie-Eve TREMBLAY**, University of Laval Québec (CA)
Microglial remodeling of neuronal circuits in the mature healthy brain

10:15-10:45 **Alain BESSIS**, IBENS Paris (FR)
Microglial control of synaptic function

10:45-11:10 *Coffee break*

11:10-11:30 Selected talk: **Thomas PFEIFFER**, IINS Bordeaux (FR)
Induction of LTP increases the motility of microglia and prolongs their dynamic contacts with dendritic spines

11:30-12:00 **Stéphane OLIET**, NCM Bordeaux (FR)
Membrane trafficking of GLT1 glutamate transporters in astrocytes

12:00-13:00 Lunch break

13:00-15:00 Poster session

Session 2 - *Chaired by Aude PANATIER and Alain BESSIS (p.m.)*

15:00-15:30 **Dmitri RUSAKOV**, University College London (UK)
Structural plasticity of synaptic environment: Insights into the machinery

15:30-16:00 **Nathalie ROUACH**, Collège de France Paris (FR)
Unraveling unconventional role for astroglial connexins in synaptic strength and memory

16:00-16:30 *Coffee break*

16:30-16:50 Selected talk: **Jonathan ZAPATA**, Collège de France Paris (FR)
Atypical neuroglial interactions at the hippocampal mossy fiber synapses

16:50-17:20 **Giovanni MARSICANO**, NCM Bordeaux (FR)
Astroglial type-1 cannabinoid receptors (CB_1) are necessary for object recognition memory and synaptic plasticity

17:20-18:30 Round table:
Brainstorming on the role of microglia and astrocytes at the synapse

19:00 -23:30 **Gala dinner** at Château Carbonnieux

Friday, October 2nd

Session 3 - **ROLE OF GLIAL CELLS IN BRAIN PATHOLOGY**
Chaired by Elena AVIGNONE and Stéphane OLIET (a.m.)

09:00-09:30 **Richard RANSOHOFF**, Lerner Research Institute Cleveland (US)
Fractalkinomics: One key to unlock the mysteries of microglia

09:30-10:00 **Michael SALTER**, University of Toronto (CA)
From receptors to pain: the molecular dynamics of pain

10:00-10:20 Selected talk: **Li TIAN**, Neuroscience Center Helsinki (FI)
Stress-Immune connection: understanding it from the systems biological perspective

10:20-10:40 *Coffee break*

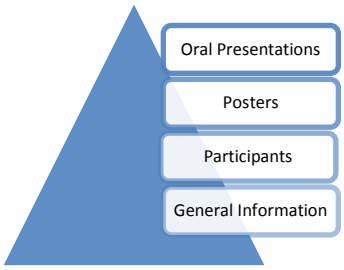
10:40-11:00 Selected talk: **Eric MARTINEAU**, University of Montréal (CA)
Alteration of glial properties at the NMJ may hinder reinnervation in ALS

11:00-11:30 **Carole ESCARTIN**, MIRCen Fontenay-aux-Roses (FR)
Functional changes in reactive astrocytes during neurodegenerative diseases

11:30-12:00 **Giorgio CARMIGNOTO**, Neuroscience Institute, National Research Council, Padova (IT)
Exploring the role of astrocytes in epileptiform activity

12:00-12:30 Closing remarks by **Christophe MULLE**

12:30 *Lunch*



INVITED SPEAKERS - IS

IS 001 Cornelius GROSS - *Microglia contribute to autism-related functional connectivity and behavioral deficits*

IS 002 Sophie LAYE - *Lipid nutrition regulates microglia activity and function: consequence on behavior*

IS 003 Etienne AUDINAT - *Microglia and maturation of synaptic circuits during cortical development*

IS 004 Beth STEVENS - *Immune mechanisms of synapse loss in health and disease*

IS 005 Marc FREEMAN - *Roles for astrocytes in neural circuit assembly*

IS 006 Richard ROBITAILLE - *Glial cells regulate synaptic properties to alter the outcome of synaptic competition at the mammalian neuromuscular junction*

IS 007 Wenbiao GAN - *In vivo studies of microglial function in synapse formation*

IS 008 Marie-Eve TREMBLAY - *Microglial remodeling of neuronal circuits in the mature healthy brain*

IS 009 Alain BESSIS - *Microglial control of synaptic function*

IS 010 Stéphane OLIET - *Membrane trafficking of GLT1 glutamate transporters in astrocytes*

IS 011 Dmitri RUSAKOV - *Structural plasticity of synaptic environment: Insights into the machinery*

IS 012 Nathalie ROUACH - *Unraveling unconventional role for astroglial connexins in synaptic strength and memory*

IS 013 Giovanni MARSICANO - *Astroglial type-1 cannabinoid receptors (CB_1) are necessary for object recognition memory and synaptic plasticity*

IS 014 Richard RANSOHOFF - *Fractalkinomics: One key to unlock the mysteries of microglia*

IS 015 Michael SALTER - *From receptors to pain: the molecular dynamics of pain*

IS 016 Carole ESCARTIN - *Functional changes in reactive astrocytes during neuro-degenerative diseases*

IS 017 Giorgio CARMIGNOTO - *Exploring the role of astrocytes in epileptiform activity*

SELECTED TALKS - ST

ST1 - Role of glial cells in brain development

ST1.1 Anne ROUMIER - *Modulation of microglia by serotonin 5-HT_{2B} receptors: possible implications for developmental synaptic refinement*

ST1.2 Joana FERREIRA - *Co-agonist tunes GluN2B-NMDA receptor trafficking and content at developing hippocampal synapses*

ST2 - Role of glial cells in brain physiology

ST2.1 Thomas PFEIFFER - *Induction of LTP increases the motility of microglia and prolongs their dynamic contacts with dendritic spines*

ST2.2 Jonathan ZAPATA - *Atypical neuroglial interactions at the hippocampal mossy fiber synapses*

ST3 - Role of glial cells in brain pathology

ST3.1 Eric MARTINEAU - *Alteration of glial properties at the NMJ may hinder reinnervation in ALS*

ST3.2 Li TIAN - *Stress-Immune connection: understanding it from the systems biological perspective*

ABSTRACTS

INVITED SPEAKERS - IS

IS 001

Microglia contribute to autism-related functional connectivity and behavioral deficits

Cornelius T. GROSS

Microglia are cells of the myeloid lineage that infiltrate the brain during development and play a role both in the maturation of brain circuits and in its response to inflammation and injury. During early development microglia phagocytosis is important for removing apoptotic neurons, while later in development it appears to have a role in selectively eliminating synapses. We and others have shown that mutations that block neuron-microglia signaling are able to interfere with this phagocytic function and result in deficits in brain wiring, although it remains unknown precisely how microglia promote synapse elimination and maturation. We have shown that mice lacking the neuron-microglia signaling chemokine Cx3cl1 show an overabundance of weak synaptic contacts associated with widespread weak functional brain connectivity and deficient social and repetitive behavior, both hallmarks of autism. These findings propose for the first time a specific neurophysiological deficit for the weak functional connectivity seen in autism and open the possibility that deficits in microglia function could contribute to key features of neurodevelopmental disorders.

IS 002

Lipid nutrition regulates microglia activity and function: consequence on behavior

LAYE Sophie
NutriNeuro, UMR Inra Univ Bordeaux 1286, LIA OptiNutriBrain Inra, Laval University

Since they were first described almost one century ago by del Rio Hortega, microglia have been appointed as the scavengers of the brain due to their peculiar and unique phagocytic function. According to the early view, microglia were thought to remain for the most part in a 'resting' state, waiting for a danger stimulus that would transform them into an 'activated' state in which they were able to phagocytose neuronal debris and dead cells. This view prevailed until modern microscopy techniques made it possible to view microglia in their 'resting' state in the living animal, at which point it was discovered that microglia are in fact highly motile in their baseline state. Microglial nomenclature has moved on from older, binary, classifications of "quiescent" and "activated" towards the recognition of microglial plasticity and of a dynamic spectrum of activity states. Groundbreaking in vivo live imaging studies in adult mouse demonstrated that microglia processes dynamically survey their environment and interact with other brain cells including neurons and astrocytes. More recent imaging studies have revealed that microglia dynamically interact with synapses where they appear to serve as ''synaptic sensors" responding to changes in neural activity and neurotransmitter release. Furthermore, several groups suggest that microglia in their resting state are able to phagocytose unwanted synapses and in this way contribute to synaptic pruning and maturation during development. Coupled with their exquisite sensitivity to pathogenic stimuli, these data suggest that microglia form a link that couples changes in brain environment to changes in brain activity.
So what environmental factors modulate microglia? What are the consequences, for the individual, of exposure to subsequent inflammatory stimuli ? What are the most sensitive period for environmental manipulation of microglia?
I will present recent evidence demonstrating that dietary lipid manipulation modulates microglia. On one hand, consumption of high fat or omega-3-deficient diets (during development or at adulthood) can trigger brain inflammation and subsequent injury in the absence of any peripheral inflammatory signaling. Conversely, lipid derivates such as resolvins are able to switch microglia for an pro-inflammatory to an anti-inflammatory status, thus restoring brain homeostasis in condition of neuroinflammation. These knowledge bring omega 3 as crucial regulators of microglia activity and bring to the clinical scene lipid nutrition in neuroinflammatory diseases.

IS 003

Microglia and maturation of synaptic circuits during cortical development

AUDINAT Etienne[1], ARNOUX Isabelle[1,2], MOSSER Coralie-Anne[1], BAPTISTA Sofia[1], HOSHIKO Maki[1,3]

[1] *Inserm U1128, Paris Descartes University, France*
[2] *Focus Program Translational Neuroscience (FTN) and Institute for Microscopic Anatomy and Neurobiology, Johannes Gutenberg University Mainz, Germany*
[3] *Graduate School of Frontier Biosciences, Osaka University, Suita, Osaka, Japan*

Besides their classical roles in pathological conditions, microglial cells are now acknowledged to interact dynamically with neurons and influence their structure and function in physiological conditions. In particular, accumulative evidence indicates that microglial cells influence the normal development of brain synapses. However, mechanisms by which these immune cells target maturating synapses and influence their functional development at early postnatal stages remain poorly understood. My presentation will be focused on the role of microglia during the development of the rodent somatosensory cortex. I will describe how microglial cells invade the whisker-related barrel field of this cortical area right after the initial steps of formation of the barrels and how they influence the functional maturation of excitatory and inhibitory cortical synapses.

IS 004

Immune mechanisms of synapse loss in health and disease

STEVENS Beth

One of the major unsolved mysteries in neuroscience is how synapses are eliminated in the healthy and diseased brain. During development, neural circuitry undergoes a remodeling process in which excess synapses are eliminated and the remaining synapses are strengthened. This pruning process is required for precise brain wiring; however the mechanisms that drive the elimination of specific synapses in the brain remain unclear. Emerging evidence implicate molecules traditionally associated with the adaptive and innate immune system. For example, recent work from our laboratory revealed a key role for microglia and molecules traditionally associated the classical complement cascade in developmental synaptic pruning. Our recent studies support a model in which 'weaker' or less active synapses in the developing brain are targeted by complement proteins (C1q, C3) and then eliminated by phagocytic microglia that express receptors for complement and other immune molecules. These findings raise the question of how microglia differentiate the synapses or axons to prune from those to leave intact. Microglia-mediated synaptic refinement appears to depend on a careful balance of "eat me" (ie. complement) and a group of novel immune- related protective signals.

An early hallmark of many neurodegenerative diseases (NDDs) is a progressive, region-specific degeneration of synapses; however, molecular mechanisms that drive synapse loss remains elusive. Our recent work suggest that aberrant activation of some of these normal immune – related pruning pathways mediate early synapse loss in neurodegenerative diseases (NDDs), including Alzheimer's Disease (AD) and Huntington's disease (HD). Understanding how these immune mechanisms drive developmental pruning may provide novel insight into how to protect synapses in NDDS and other disorders of synaptic dysfunction, including autism and schizophrenia.

IS 005

Roles for astrocytes in neural circuit assembly

FREEMAN Marc R

Department of Neurobiology/Howard Hughes Medical Institute, University of Massachusetts Medical School, Worcester, MA USA

Astrocytes are key regulators of neural circuit assembly and function, but the molecular basis of neuron-astrocyte signaling events remain poorly defined in any organism. We recently made the exciting discovery that astrocytes are present in the *Drosophila* larval and adult nervous system. This provides us with the opportunity to exploit the array of powerful molecular-genetic approaches available in flies to explore neuron-astrocyte interactions in vivo. This presentation will cover our current understanding of the cell biology of *Drosophila* astrocytes, how they modulate the functional assembly and pruning of specific neural circuits, and our molecular understanding of these interactions. It is becoming increasingly clear that *Drosophila* astrocytes share a high degree of molecular and functional similarities with mammalian astrocytes. Based on the remarkable success with which invertebrates were used to help dissect the cell biology of the neuron, invertebrate models like *Drosophila* appear well positioned to help rapidly unravel fundamental aspects of glial cell development and function.

IS 006

Glial cells regulate synaptic properties to alter the outcome of synaptic competition at the mammalian neuromuscular junction

ROBITAILLE Richard[1,2], DARABID Houssam[1,2]

[1] *Département de neurosciences, Université de Montréal*
[2] *Groupe de recherche sur le système nerveux central*

The precise wiring of synaptic connections is shaped by elimination of supernumerary inputs competing for the innervation of the same target cell. At the neuromuscular junction (NMJ), this competition depends on the synaptic efficacy of competing terminals such that one input is strengthened while others are weakened and eventually leading to their elimination. Despite the importance of Perisynaptic Schwann Cells (PSCs), glial cells at the NMJ, in the modulation of synaptic efficacy and plasticity at adult NMJs, their role during synaptic competition remains unclear. Hence, the goal of this work was to study the differential modulation of weak and strong nerve terminals by PSCs and determine the impact on the outcome of synaptic competition.

We performed intracellular recordings from dually innervated P7-8 mouse Soleus muscle fibers to assess synaptic activity and monitored PSC activity using confocal Ca^{2+} imaging. PSCs were loaded with the Ca^{2+} indicator Fluo-4 and the morphological dye Alexa 594 using single cell electroporation. PSCs decode synaptic competition as revealed by tight relationship between the size of Ca^{2+} responses and the synaptic strength of each input (i.e. weak input generated smaller Ca^{2+} responses than the strong one). At the same NMJ, the strong input showed a long-lasting potentiation of neurotransmission while the weak one displayed only a transient and smaller potentiation. To determine whether the differential plasticity of competing terminals was related to PSCs Ca^{2+} responses, single PSCs were loaded with Diazo-2, photoactivable BAPTA molecule. PSCs activity was blocked by photoactivation of Diazo-2 using 405 nm laser light. Photoactivation of Diazo-2 blocked PSCs Ca^{2+}-responses and abolished the persistent plasticity of the strong inputs and greatly reduced the short term plasticity. Furthermore, direct induction of large (mimicking activation by strong inputs), but not small Ca^{2+} responses with the Ca^{2+} caged compound NP-EGTA resulted in a long-lasting potentiation of the strong input while the weak terminal displayed only a transient one. These results suggest that PSCs activity is both necessary and sufficient for the expression of the differential plasticity of competing nerve terminals. Finally, the in vivo period of synaptic competition was extended when the ability of PSCs to decode synaptic competition was blocked with the P2Y1 receptor antagonist MRS2179. This suggests that the differential regulation by PSCs of strong and weak inputs influences the outcome of synaptic competition and elimination.

IS 007

In vivo studies of microglial function in synapse formation

Christopher N. Parkhurst[1], Lianyan Huang[2], Joseph Cichon[1], Sally Levinson[1], Mirko Santello[1], Elina Shtridler[1], Ipe Ninan[3], Juan J. Lafaille[4], Barbara L. Hempstead[5], Dan R. Littman[4, 6], Guang Yang[2], and **Wenbiao GAN**[1]

[1] Skirball Institute, New York University School of Medicine, New York, NY, 10016
[2] Department of Anesthesiology, New York University School of Medicine, New York, NY, 10016
[3] Department of Psychiatry, New York University School of Medicine, New York, New York 10016
[4] Skirball Institute, Department of Pathology, New York University School of Medicine, New York, NY, 10016
[5] Department of Medicine, Weill Cornell Medical College, New York, NY, 10065
[6] Howard Hughes Medical Institute, New York University School of Medicine, New York, NY 10016, USA

Microglia are the resident macrophages of the central nervous system and their functions have been extensively studied in various brain pathologies. The physiological roles of microglia in brain plasticity and function remain unclear. We have generated CX_3CR1^{CreER} mice expressing tamoxifen-inducible Cre recombinase that allow for specific manipulation of gene function in microglia. Using CX_3CR1^{CreER} to drive diphtheria toxin receptor expression in microglia, we found that microglia

could be specifically depleted from the brain upon diphtheria toxin administration. Mice depleted of microglia show deficits in multiple learning tasks and a significant reduction in motor learning-dependent synapse formation. Furthermore, Cre-dependent removal of brain-derived neurotrophic factor (BDNF) from microglia recapitulated some of the effects of microglia depletion. These results reveal important physiological functions of microglia in learning and memory by promoting learning-related synapse formation through BDNF signaling. We are currently investigating the role of microglia and microglial BDNF signaling in regulating synaptic plasticity in mouse models of chronic pain and traumatic brain injury.

IS 008

Microglial remodeling of neuronal circuits in the mature healthy brain

TREMBLAY Marie-Ève[1,2]

[1] *Axe Neurosciences, Centre de recherche du CHU de Québec, Québec City, Canada*
[2] *Département de médecine moléculaire, Université Laval, Québec City, Canada*

This past decade has witnessed a revolution in our understanding of microglia, especially since their roles in the healthy brain have started to unravel. These cells were shown to actively maintain health, in concert with neurons and other types of glial cells, providing further insight into their crucial involvement with diseases.
To set microglia on the stage, I will begin my talk by explaining briefly who they are, what they do in health and disease, and how they can be studied using non-invasive imaging approaches. I will also introduce microglia-synapse interactions and their emerging roles in the experience-dependent remodeling of neuronal circuits during normal physiological conditions.
The body of my talk will be dedicated to the significance of these new roles in the healthy brain and one underlying molecular mechanism: signalling between the chemokine fractalkine (CX3CL1) mainly expressed by neurons and its unique receptor CX3CR1 exclusively found on microglia. In particular, I will present our recent work showing that this pathway regulates microglial phenotype, microglia-synapse interactions, synaptic plasticity and function, and the behavioural outcome following chronic unpredictable stress, an environmental challenge associated with a massive remodeling of neuronal circuits and elimination of synapses.
Lastly, I will present a possibly new microglial phenotype: the mysterious “dark” cells, which extensively interact with synapses in various contexts of health and disease.

IS 009

Microglial control of synaptic function

BESSIS Alain[1,2,3]

[1] *Institut de Biologie de l'Ecole Normale Supérieure, F-75005 Paris, France;*
[2] *Institut National de la Santé et de la Recherche Médicale U1024, F-75005 Paris,*
[3] *Centre National de la Recherche Scientifique, Unité Mixte de Recherche 8197, F-75005 Paris*

Microglia are now recognized as genuine partners of several neuronal functions in the healthy brain. However, whereas their roles related to their phagocytic activity are well studied, their involvement in information processing has somehow been overlooked. Yet, microglial cells are highly dynamic and react rapidly to the modifications of their environment. They express membrane receptors for all known neurotransmitters and are thus able to sense neuronal activity. We have now studied the involvement of microglia in the control of both excitatory and inhibitory neurotransmission.
We previously showed that stimulation of microglia induces a rapid production of ATP that induces glutamate release from astrocytes. This glutamate then increases the EPSC frequency through neuronal mGluR5. We recently explored how microglia also regulate inhibitory neurotransmission. We have depleted and stimulated microglial cells and demonstrated that these cells achieve a tonic as well as a short-term control of spontaneous glycinergic but not gabaergic synaptic events associated with a specific regulation of the synaptic accumulation of glycine (GlyR) but not $GABA_A$ receptors. Single particle tracking experiments demonstrated that selective modulation of the diffusion and synaptic stabilization of $GABA_AR$ and GlyR drive this differential accumulation at synapses and tune an adaptive activity-dependent regulation of synaptic GlyR. In conclusion, our results describe microglia as fulltime actors of the regulation of synaptic transmission.

IS 010

Membrane trafficking of GLT1 glutamate transporters in astrocytes

OLIET Stéphane[1,2]

[1] *Neurocentre Magendie, Inserm U862, Bordeaux, France*
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Glutamate is the major excitatory transmitter in the brain. Its concentration in the synapse and its diffusion in the extracellular space are tightly controlled by the astroglial transporter GLT-1. These transporters ensure point-to-point transmission and prevent excessive accumulation of glutamate that could be toxic to neurons. Although data are available on the regulated expression of these proteins in physiological and pathological conditions, as well as on its intracellular trafficking,

nothing is know about its membrane dynamics. Using quantum dot imaging of single GLT-1 transporter, we observed that not only GLT-1 is highly mobile at the surface of astrocytes, but that this mobility depends strongly on neuronal and synaptic activity as well as on the activity of the transporter itself. Furthermore, GLT-1 membrane diffusion slows down dramatically at the vicinity of synaptic contacts, suggesting that an active process is engaged when the transporter senses glutamate. Finally, reducing experimentally GLT-1 membrane trafficking through cross-linking with anti-bodies modifies significantly the kinetics of the glutamatergic synaptic currents. These results provide new insights on the astroglial glutamate transporter GLT-1 and the processes by which it shapes excitation and impacts synaptic transmission. It also offers open the door to new strategies to tackle neuronal disorders involving GLT-1 dysfunctions.

IS 011

Structural plasticity of synaptic environment: Insights into the machinery

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Memory formation in the brain is thought to rely on the remodelling of synaptic connections which ultimately leads to neural network rewiring. This process is likely to involve astroglial protrusions occurring in the vicinity of excitatory synapses. Indeed, recent evidence has associated astroglial Ca^{2+} activity with diverse molecular signals influencing functional synaptic connectivity. The phenomenology, cellular mechanisms and causal relationships of use-dependent astroglial restructuring remain however poorly understood. To monitor rapid nanoscopic rearrangement of astroglia upon induction of long-term potentiation (LTP), we combined electrophysiology with two-photon excitation microscopy and photolytic uncaging. We document NMDA receptor dependent-withdrawal of astroglial processes from the vicinity of synapses following LTP induction, both at the level of synaptic populations and at the level of individual potentiated synapses. This reduction in synaptic astroglial coverage facilitates escape of synaptic glutamate thus boosting NMDA receptor-mediated cross-talk among synapses. The molecular mechanisms behind astroglial restructuring require local Ca^{2+} elevations but do not appear to involve mGluRs or IP_3-receptor signalling. Experiments are underway to build a conceptual understanding of the underlying molecular interactions acting within the microscopic vicinity of synapses.

IS 012

Unraveling unconventional role for astroglial connexins in synaptic strength and memory

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Astrocytes play active roles in brain physiology by dynamic interactions with neurons. Connexin 30, one of the two main astroglial gap-junction subunits, is thought to be involved in behavioral and basic cognitive processes. However, the underlying cellular and molecular mechanisms are unknown. We show here in mice that connexin 30 controls hippocampal excitatory synaptic transmission through modulation of astroglial glutamate transport, which directly alters synaptic glutamate levels. Unexpectedly, we found that connexin 30 regulated cell adhesion and migration and that connexin 30 modulation of glutamate transport, occurring independently of its channel function, was mediated by morphological changes controlling insertion of astroglial processes into synaptic clefts. By setting excitatory synaptic strength, connexin 30 plays an important role in long-term synaptic plasticity and in hippocampus-based contextual memory. Taken together, these results establish connexin 30 as a critical regulator of synaptic strength by controlling the synaptic location of astroglial processes.

IS 013

Astroglial type-1 cannabinoid receptors (CB_1) are necessary for object recognition memory and synaptic plasticity

Giovanni MARSICANO

Astrocytes express a wide variety of G protein-coupled receptors (GPCR) that can influence cognitive functions such as learning and memory. Cannabinoids and endocannabinoids modulate memory processes through the GPCR CB_1 receptor. Interestingly, similarly to neurons, astrocytes express functional CB_1 receptors capable of modulating the effects of exogenously administered cannabinoid agonists on hippocampal synaptic plasticity and working memory. However, the endogenous roles of astroglial CB_1 receptor in long-term memory and synaptic plasticity remain unknown.
Here, we show that the conditional genetic deletion of CB_1 receptor from astrocytes (GFAP-CB_1-KO mice) impairs both *in vitro* and *in vivo* hippocampal long-term potentiation (LTP) induction and long-term object recognition memory. Notably, administration of D-serine, a gliotransmitter that is a co-agonist at the N-methyl-D-Aspartate receptor (NMDAR), restores the long-term memory deficit and the induction of LTP in mutant mice. Finally, GFAP-CB_1-KO mice present strongly reduced occupancy of the co-agonist binding site of NMDARs. Thus, astroglial CB_1 receptors are necessary for long-term memory and the induction of NMDAR-dependent LTP, via the modulation of the occupancy of the NMDAR co-agonist binding site. This study reveals an unexpected role for astroglial CB_1 receptors in neural information processing and memory formation.

IS 014

Fractalkinomics: One key to unlock the mysteries of microglia

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Microglia arise during primitive hematopoiesis and enter the developing murine brain rudiment around E10.5, before other glia and well in advance of definitive specification of neuronal populations and networks. During development, microglia interact extensively with neurons, helping to establish neuronal populations through influences on survival, apoptosis and corpse-clearance. As synapses form during early postnatal life, microglia refine neuronal network properties by synaptic pruning.

Neuron-microglial and microglial-neuron dialogs are mediated by contact-dependent and soluble signals. Fractalkine (CX3CL1) is implicated in both forms of signaling. In particular, fractalkine is expressed as a transmembrane chemokine expressed in the CNS solely by neurons, which also release soluble fractalkine to signal at a distance. The fractalkine receptor (CX3CR1) is restricted to microglia among CNS cells. Perturbation of fractalkine/fractalkine receptor signaling results in altered microglial function, with consequences observed from mid-gestation through aging and neurodegeneration. Using mice deficient for fractalkine or its receptor provides a wealth of insights into microglial function in the intact CNS and may have direct clinical relevance, given a common hypomorphic CX3CR1 allele in humans. Examination of the mechanism underlying fractalkine regulation of microglial responses may yield information useful for translating model results to clinical application.

IS 015

From receptors to pain: the molecular dynamics of pain

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Neuron-microglial interactions are increasingly recognized as being key for physiological and pathological processes in the central nervous system. Microglia have been found to play a causal role in neuropathic pain behaviours resulting from peripheral nerve injury, and a core neuron-microglia-neuron signaling pathway has been elucidated. Within the dorsal horn, microglia suppress neuronal inhibition by a cascade involving activation of microglial P2X4 receptors causing the release of brain derived neurotrophic factor (BDNF). BDNF acts on trkB receptors which leads to a rise in intracellular chloride concentration in dorsal horn nociceptive output neurons, transforming the response properties of these neurons. In addition to suppressing inhibition, peripheral nerve injury causes activity-dependent facilitation at dorsal horn glutamatergic synapses which enhances nociceptive transmission. This enhancement is mediated by intracellular signaling networks involving serine/threonine and tyrosine kinases within nociceptive transmission neurons. Key for this enhancement is facilitation of NMDA receptor function by Src family tyrosine kinases. Recently we have discovered that microglia-to-neuron signaling is not only critical for pain hypersensitivity after peripheral nerve injury but also for the paradoxical hyperalgesic effect of morphine and other opioids. We anticipate that by targeting microglia-neuron signaling pathways new therapeutic strategies for chronic pain as well as its comorbid sequelae may be developed.

Funding: Supported by CIHR, Krembil Fdn, CRC and Anne and Max Tanenbaum Chairs.

IS 016

Functional changes in reactive astrocytes during neurodegenerative diseases

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Astrocytes become reactive in response to virtually all pathological situations in the brain. Astrocyte reactivity is characterized by classical features such as cellular hypertrophy and overexpression of intermediate filament proteins. But, how the supportive functions of astrocytes are altered by their reactive state is largely unknown. In fact, controversial data on the contribution of reactive astrocytes to neurodegenerative diseases (ND) have been generated in the last years. Such conflicting results may stem from the use of disparate models of astrocyte reactivity and poorly selective methods to manipulate reactive astrocytes *in situ*. Here, we aimed to better delineate the roles of reactive astrocytes during ND, in relevant *in vivo* models and using cell-specific approaches.
We identified the JAK2/STAT3 pathway as a universal signaling cascade responsible for astrocyte reactivity in various ND models. We then developed viral vectors that modulate the JAK/STAT3 pathway specifically in astrocytes *in vivo*. We found that overexpression of the inhibitor SOCS3 in reactive astrocytes of mouse models of Alzheimer's and Huntington's diseases was sufficient to revert them to a resting-like phenotype and to decrease microglial activation. It is then possible to assess how blocking astrocyte reactivity impacts disease outcomes in relevant animal models of ND. In particular, this selective approach to manipulate astrocyte reactivity i*n situ* will help better delineate the influence of reactive astrocytes at the synapse during ND.
Financial support: ANR JCJC 1402-1

IS 017

Exploring the role of astrocytes in epileptiform activity

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Epilepsy is a brain disorder characterized by unpredictable episodes of uncontrolled seizures. At a cellular level, seizure-like ictal events manifest as synchronous, intense firing discharges involving large populations of neurons. Our knowledge of the mechanisms underlying the initiation, the propagation and the cessation of focal seizures is, however, unsatisfactory. Using an experimental model of focal epilepsy in enthorinal or temporal cortex slice preparations, we recently revealed a

contribution of astrocytes to the generation of focal ictal discharges. We found that in a cortical neuronal network prone to seizures due to slice perfusion with 4-aminopyridine and low Mg^{2+}, an episode of neuronal hyperactivity triggered by local NMDA applications, evoked metabotropic glutamate receptor-mediated Ca^{2+} elevations in astrocytes which lowered seizure threshold. At the basis of this astrocyte action is probably the release of glutamate which enhances synchronous activities in pyramidal neurons. Because focal ictal discharge propagation is governed by a feed-forward inhibition mainly generated by parvalbumim fast-spiking (Pv-FS) interneurons (Trevelyan et al., 2006, 2007; Cammarota et al. 2013), we advance the hypothesis that this action of GABAergic interneurons can be also affected by astrocyte signaling. In favour of this hypothesis, a large number of astrocytes from both the enthorinal and temporal cortex were found to respond to local GABA applications with repetitive GABAB-mediated Ca^{2+} elevations. Activation of individual Pv-FS interneurons by intracellular injections of depolarizing current pulses also triggered Ca^{2+} elevations and gliotransmitter release from nearby astrocytes which activated NMDAR-mediated slow inward currents in Pv-FS interneurons. Astrocytic glutamate can have on these GABAergic interneurons a dual action that may ultimately: i) oppose seizure generation/spread; ii) favour hyperactivity (by enhancing neuronal synchrony or the depolarizing, excitatory effects of GABA). Clarification of astrocyte-interneuron reciprocal signaling may represent an important step towards a better understanding of the cellular events at the basis of generation, propagation and cessation of focal ictal discharges.

SELECTED TALKS - ST

ST1.1

Modulation of microglia by serotonin 5-HT$_{2B}$ receptors: possible implications for developmental synaptic refinement

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In physiological conditions, microglia were shown to modulate synapse formation and activity, and thus to shape neuronal circuits. However, mediators controlling the activity of microglia in order to modulate their functions remain elusive. Here, we tested the hypothesis of cross-interactions between serotonin and microglia.
We first established that the 5-HT$_{2B}$ receptor is the main serotonin receptor expressed by postnatal microglia *in vivo*. We also showed that microglia processes are in close apposition with serotonergic varicosities suggesting that microglia can sense serotonin. Then, using two-photon microscopy on acute brain slices and local delivery of serotonin, we found that microglial processes can move rapidly towards a source of 5-HT in Htr2B+/+ mice, but not in Htr2B−/− mice lacking the 5-HT$_{2B}$ receptor.These results clearly show that 5-HT via 5-HT$_{2B}$ receptors can modulate microglial process motility.
We then investigated whether the *in vivo* model of maturation of retinal projections to the thalamus, a developmental step known to be controlled both by serotonin and by microglia, could be impaired by microglia insensitivity to serotonin. We observed that *Htr$_{2B}^{-/-}$* mice present specific anatomical alterations of the ipsilateral projecting area of retinal axons into the thalamus. Finally, expression of activation markers are altered in microglial cultures from *Htr$_{2B}^{-/-}$* compared to wild type ones.
In conclusion, our results support the hypothesis that serotonin interacts with microglia through the 5-HT$_{2B}$ receptor and that these interactions could participate in postnatal brain maturation.

ST1.2

Co-agonist tunes GluN2B-NMDA receptor trafficking and content at developing hippocampal synapses

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Activation of NMDA receptor (NMDAR) requires the binding of glutamate and co-agonist, such as glycine or D-serine. These co-agonists also influence NMDAR signaling by modulating the receptor trafficking in a GluN2 subunit-dependent manner. Since the synaptic maturation is characterized by a switch from GluN2B-rich to GluN2A-rich NMDAR, we here explored the possibility that glycine and D-serine synaptic availability throughout development is instrumental in driving the GluN2-NMDAR switch. Using electrophysiological and single nanoparticle imaging approaches, we report that glycine is the endogenous co-agonist at CA3-CA1 synapses early after birth and is gradually replaced by D-serine, a switch parallel to the one of GluN2B- to GluN2A-NMDAR. Strikingly, exogenous and endogenous acute manipulations of glycine levels altered the surface distribution and dynamics of synaptic GluN2B-NMDAR but not GluN2A-NMDAR. Together, these data demonstrate that glycine, the main co-agonist early in development, tunes synaptic GluN2B-NMDAR trafficking and hence NMDAR signaling in maturing hippocampal synapses.

ST2.1

Induction of LTP increases the motility of microglia and prolongs their dynamic contacts with dendritic spines

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In the healthy brain microglia continuously scan their surroundings by rapidly extending and retracting their highly ramified processes. During this scanning activity microglia establish transient contacts with synapses in an activity-dependent way. Microglia can sense changes in neuronal activity and their processes are steered towards active neurons, a phenomenon which may be mediated through the activation of neuronal NMDARs. Furthermore, recent work has implicated microglia in synapse remodeling during development and experience-dependent plasticity, giving rise to the idea that microglia might play a role in synaptic plasticity.
However, very little is known about how microglia associate with synapses during basal synaptic transmission, and whether microglia can sense and respond to the induction of synaptic plasticity. In this study, we investigated these questions using a combination of two-photon time-lapse imaging and electrophysiological recordings in acute hippocampal brain slices from double transgenic mice, where microglia and neurons are fluorescently labeled. We analyzed the scanning motility of microglia and their physical interactions with dendritic spines of CA1 pyramidal neurons before and after the induction of hippocampal LTP using electrical high-frequency stimulation (HFS).
Our analysis reveals that at a given time point only a very small fraction of dendritic spines were contacted by a microglial process. However, due to their high motility the total number of contacted synapses grew substantially over time. Contacts between microglial processes and dendritic spines were brief during basal synaptic transmission. After LTP induction microglial scanning motility was enhanced, while contact duration was prolonged and their number reduced. The application of the NMDAR antagonist APV prevented the HFS-induced changes in microglial scanning behavior and microglia-spine interactions. The elevated microglial motility and microglia-synapse contact stability during LTP corroborate the idea that microglia contribute to activity-dependent remodeling of synapses in the healthy mature brain. We have started to apply super-resolution STED microscopy to investigate the dynamic interactions between microglial processes and dendritic spines at the nanoscale in acute brain slices. Together with electrophysiology and two-photon glutamate uncaging, the super-resolution approach will allow us to study the involvement of microglia in synaptic plasticity at the level of single spines

ST2.2

Atypical neuroglial interactions at the hippocampal mossy fiber synapses

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Astrocytes play an important role in synaptic function through multiple mechanisms including structural isolation of synapses, active removal of neurotransmitters and ions as well as release of gliotransmitters. Neuroglial interactions and their critical contribution to synaptic transmission have been characterized in many brain regions, including hippocampal areas CA1 and dentage gyrus (DG). One particular synapse though is formed by the projection of DG granule cells axons onto CA3 pyramidal neurons and is of high importance as it represents the primary inputs of information into the hippocampal trisynaptic circuit. The so called mossy fiber synapses (MFS) are unique structures present as giant boutons enclosing several active zones isolated from extrasynaptic areas but exposed to spillover within the terminal. Such singular morphology translates into particular physiological properties, showing notably a highly dynamic plasticity. The role of astrocytes at the MFS however remains elusive. We thus investigated the structural and functional interactions between *stratum lucidum* hippocampal CA3 astrocytes and MFS, and their involvement in the dynamic properties of MFS. We first assessed astroglial synaptic coverage, and found that astrocytes interactions with MFB are much more restricted than typical Schaffer-collateral - CA1 synapses. We then explored how astrocytes manage to endorse ionic regulation in this region. To this end, we investigated Ca^{2+} signaling in CA3 astrocytes and its impact on basal synaptic transmission and short-term plasticity. We found that basal Ca^{2+} fluctuations were only partly activity-dependent and that calcium rise was not concomitantly elicited by evoked short-term plasticity patterns. Surprisingly, calcium signaling in astrocytes did not significantly affect mossy fibers short-term plasticities. Besides, we also recorded synaptically-evoked astroglial currents and found that they are mainly composed of a long-lasting potassium current sensitive to neuronal activity and principally mediated by $K_{ir}4.1$ channels. Remarkably, these astroglial $K_{ir}4.1$ channels contribute to activity-dependent mossy fibers short-term plasticities. Altogether this study brings new insights on the role of CA3 astrocytes in information processing at a highly dynamic synapse, and may contribute to identify novel targets in pathologies involving learning, memory dysfunction and psychiatric disorders.

ST3.1

Alteration of glial properties at the NMJ may hinder reinnervation in ALS

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Amyotrophic lateral sclerosis (ALS) is a fatal neurodegenerative disease characterized by the loss of both upper and lower motoneurons. Destruction of the neuromuscular junctions (NMJ) occurs before disease onset, supporting the notion that ALS may have a long silent presymptomatic phase. Over the last decade, evidence of neurodegeneration being mediated by an abundance of non-cell autonomous processes has highlighted the importance of glial cells in this disease. However, the contribution of Perisynaptic Schwann cells (PSCs), glial cells at the NMJ, is still ill-defined in ALS despite their involvement in the synaptic and structural plasticity of healthy NMJs.
In the *SOD1*^{G37R} mouse model, we previously reported an alteration of PSC properties in the partially disease-resistant *Soleus* muscle. These changes appeared at a presymptomatic (P120) stage of the disease, persisted through later stages and were based on an over-activation of PSCs muscarinic receptors (mAChRs), which are known to maintain PSCs in a state of maintenance. Since adequate decoding of synaptic activity and reduction of mAChR activation are required for PSCs to adopt a pro-regenerative (repair) phenotype, we hypothesize that PSCs may not respond adequately to denervation in ALS. However, if that is the case, mAChR overactivation should also be present in more severely affected fast-twitch muscles, such as the *Sternomastoid* muscle.
Hence, we first tested whether PSC properties were also altered in the *Sternomastoid* using Ca2+ imaging on isolated nerve muscle preparation. We found that glial Ca2+ responses to endogenous neurotransmitter release evoked by motor nerve stimulation were greatly diminished at P180 in *SOD1*^{G37R} animals. In line with our hypothesis, PSCs mAChR-dependent decoding was nevertheless increased in the *Sternomastoid* as revealed by selective pharmacological agonists and antagonists.
Second, we evaluated pro-regenerative properties of PSCs. In particular, PSCs on denervated NMJs should phagocytose axonal debris and extend processes toward innervated NMJs, guiding sprouts of nerve terminals back to the denervated NMJ. Consistent with a misregulated repair capability in the *Soleus* and *Sternomastoid* muscles, PSCs on denervated NMJs of symptomatic *SOD1*^{G37R} animals failed to upregulate Mac-2, a marker of glial phagocytosis, while PSCs on innervated NMJs upregulated it. Furthermore, PSCs extended disorganized processes from denervated NMJs, consequently impairing nerve terminal sprouting. Together, these results suggest that neuron-glia communication at the NMJ is ubiquitously altered early in ALS, which seem to prevent PSCs from adopting a pro-regenerative phenotype, thus resulting in a chaotic glial response to denervation.

ST3.2

Stress-Immune connection: understanding it from the systems biological perspective

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Accumulating evidence suggests that abnormal proinflammatory activation of innate and adaptive immune cells can be detrimental for neurogenesis and affect synaptic formation and neurotransmission. Animal studies have provided convincing evidence on the role of immune genes in regulating synaptic neurotransmission and plasticity, metabolism of neurotransmitters and neural growth factors, neurogenesis, and connectivity of brain circuits that underlie cognition and emotion. And new therapies based on the anti-inflammatory strategy to treat these diseases have started to emerge in clinics. Such progress encourages for a more intensive and deeper research on the immune-mediated mechanism at the molecular levels that underlie neuropsychiatric diseases. The advent of the cutting-edge sequencing technology allows us to systemically identify susceptibility genes that contribute to pathogenesis of diseases and to evaluate responses of treatments. Furthermore, genetic and gene expression studies, in humans and animal models of psychiatric disorders, are becoming increasingly integrated. My group has the expertise in studying the mechanisms of immune activation in animal models of neuropsychiatric and neurological diseases by molecular and cellular biological approaches. We focus on studying the role of immune activation in controlling the brain development, behaviors and in neuropsychiatric diseases with both schizophrenic patients and animal models, and use the cutting-edge systems biological and laboratorial approaches to holistically evaluate the role of immune-related genes in neuropsychiatric diseases in both clinical and preclinical aspects. The results of our studies potentially allow a deeper understanding of the immune-mediated mechanisms in the pathophysiology of neuropsychiatric diseases and help develop novel mechanism-based prognostic tools for a more efficacious treatment for the cognitive deficits in schizophrenic patients.

1. XiuMH., Lin CG., **Tian L.**, Tan YL., Chen J., Chen S., Tan SP., Wang ZR., Yang FD., Chen DC., and Zhang XY. (**2015**) Increased IL-3 serum levels in chronic patients with schizophrenia: Associated with psychopathology. ***Psychiatry Res.*** DOI: 10.1016/j.psychres.2015.07.029
2. Ma L., Piirainen S., Kulesskaya N., Rauvala H., and **Tian L.*** (**2015**) Association of brain immune genes with social behavior in inbred mouse strains. ***J. Neuroinflam.*** 10.1
3. 186/s12974-015-0297-5
4. Ma L., Kulesskaya N., Voikar V., and **Tian L.*** (**2015**) Differential expression of brain immune genes and schizophrenia-related behavior in C57BL/6N and DBA/2J female mice. ***Psychiatry Res.*** DOI: 10.1016/j.psychres.2015.01.001
5. **Tian L.**, Tan YL., Chen DC., Lv MH., Tan SP., Soares JC., and Zhang XY. (**2014**) Reduced serum TNF alpha level in chronic schizophrenia patients with or without tardive dyskinesia. ***Prog. Neuropsychopharm. & Biol. Psych.*** 54: 259-64
6. Li ZL., Ma L., Kulesskaya N., Voikar V., and **Tian L.*** (**2014**) Microglia are polarized to M1 type in high anxiety inbred mice in response to lipopolysaccharide challenge. ***Brain Behav. Immun.*** 38: 237-48
7. **Tian L.**,* Rauvala H., and Gahmberg CG. (**2009**) Regulation of CNS immune responses by neurons. [Review] ***Trends in Immunol.*** 30 (2): 91-9

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ABSTRACTS

P1.1

Involvement of Fractalkine/CX3CR1 signaling in the synaptic development of hippocampal circuit in mice

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Microglia are the resident immune cells of CNS. They have been traditionally studied in contexts of trauma, injury and disease, but in the last few years a series of discoveries have started to unravel their active involvement in normal CNS function. Microglia are able to establish dynamic interactions with synapses during brain development and in adulthood, regulating neuronal circuit maturation, refinement and plasticity. Thus, defective microglia models are a useful tool to investigate the role of microglial cells in synaptic development. In particular, the CX3CR1 KO mouse model, in which neuron-microglia interaction is altered, is characterized by impaired brain connectivity that persists in adulthood. The EPSC properties observed in these mice reveal a reduction in synaptic multiplicity, associated to abnormal features of synaptic boutons, and this may reflect an impaired hippocampal synaptic pruning.
We further investigate the role of Fractalkine/CX3CR1 axis in the development of hippocampal circuits, to verify whether synaptic defects detected in CX3CR1 KO mice may also reflect abnormal functional properties of synaptic connections. Patch clamp recordings on CA1 pyramidal neurons were performed in different conditions of release probability, obtained manipulating the $[Ca^{2+}]/[Mg^{2+}]$ ratio.
Our data confirm that hippocampal CA3-CA1 synapses of CX3CR1 KO mice have reduced functional connectivity, as demonstrated by stimulation/response curves. The study of evoked synaptic currents show that the defective connectivity of CX3CR1 KO mice could be due to a lower release probability. This hypothesis is supported by a higher failure rate, displayed by KO synapses, showing, in opposite, normal paired pulse facilitation and potency.

Consistently, in conditions of high release probability differences observed between WT and CX3CR1 KO mice disappear. In fact, in this condition, the number of failures in the KO mice are decreased and the two genotypes have similar failure rate. In addition, the study of spontaneous and miniature EPSC amplitude reveals typical synaptic multiplicity in CX3CR1 KO mice, pointing to a functional defect.
These results suggest that the disruption of neuron-microglia interaction mediated by Fractalkine/CX3CR1 axis leads to abnormal features of excitatory hippocampal synapses in CX3CR1 KO mice, relying on reduced release probability.

P1.2

The role of microglial cells in the development of neuronal networks

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Objective of this study is to investigate the role of microglia into the development and maturation of GABAergic network.
Microglial cells colonize the central nervous system since embryonic stages, when they start to interact with other element of the central nervous system by sensing and responding to a variety of signals in the environment. A privilege communication pathway between microglia and neurons involves Fractalkine (CX3CL1), a chemokine released only by neurons and specifically recognized by microglial receptors CX3CR1. In the hippocampus, disruption of this signal induces alteration of microglia colonization as well perturbations of development of glutamatergic transmission during the second postnatal week. However the role of microglia on neuronal activity during the first postnatal week is unknown. In particular nothing is known on GABAergic activity that, contrary to mature system, is excitatory during this period. The activity in the hippocampus during the first post-natal week is characterized by Giant Depolarizing Potentials (GDPs). This synchronous network activity decreases its frequency and disappears towards the end of the first post-natal week. It is mediated by glutamate and GABA, and it plays a role in development and maturation of neuronal connections.
In this study we investigate the role of the signaling fractalkine pathway on the GABAergic signal during the ten first postnatal days and on the microglial colonization of hippocampus. To this aim we quantify the number of microglia from P0 (postnatal day zero) to P15 in several sub-regions of hippocampus in CX3CR1 knock-out (KO) mice and compared to heterozygous and wild-type (WT) animals. In parallel, we characterized the hippocampal neuronal activity by patch clamp recording. Our results show that there is a reduction in the density of microglial cells since the first post-natal week in the stratum oriens but not in the stratum radiatum, where a lower density is observed

starting from P9. By recording spontaneous activity in CA3 pyramidal neurons, we observed a delay in the disappearance of GDPs in KO mice, since contrary to WT they persist at P9. In contrast, any difference in amplitude or frequency of spontaneous pharmacologically isolated GABAergic activity was observed at the same time window.
Thus the disruption of the specific neuronal-microglia signaling pathway on one hand impacts the microglia colonization of the hippocampus and on the other hands affects specifically neuronal network activity during a time window critical for the establishment of neuronal connections.

P1.3

Glial cells control synaptic activity and connectivity during the development of mammalian neuromuscular junctions

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The precise wiring of synaptic connections requires the elimination of supernumerary inputs competing for the innervation of the same target cell during postnatal development. At the neuromuscular junction (NMJ), this competition depends on the synaptic activity of competing terminals which leads to the strengthening of one input whilst others are weakened and eventually eliminated. Little is known about the mechanisms responsible for this strengthening and weakening of competing terminals. Moreover, the role of glial cells during synaptic competition remains ill-defined despite their known importance in the modulation of synaptic activity at adult NMJs. Here, we propose that perisynaptic Schwann cells (PSCs), the glial cells at NMJs, play a key role in synapse elimination and connectivity by regulating the synaptic activity of competing nerve terminals.
We performed intracellular recordings from dually innervated P7-8 mouse *Soleus* muscle fibres and monitored PSC activity using confocal Ca^{2+}imaging. We observed a strong relationship between the size of PSCs Ca^{2+}responses and the synaptic efficacy of competing inputs. Moreover, at the same NMJ, the strong input showed a long-lasting potentiation of neurotransmission while the weak input displayed only a small transient potentiation. This differential plasticity of competing terminals depends on PSCs Ca^{2+} signalling. Indeed, inhibiting PSCs Ca^{2+} activity, by either the photoactivation of Diazo-2 (photoactivable BAPTA loaded into PSCs by single cell electroporation), or by the blockade of PSCs purinergic P2Y1 receptors, blocked PSCs Ca^{2+}-responses and resulted in the prevention of synaptic plasticity. In addition, the controlled raise of Ca^{2+} in PSCs, using the caged-compound NP-EGTA, was sufficient to induce synaptic plasticity. Finally, chronic in vivo blockade of PSCs P2Y1 receptors resulted in an increased proportion of poly-innervated NMJs and delayed synapse elimination.
Altogether, these data indicate that glial cells regulate synaptic plasticity in a Ca^{2+}-dependent manner which may influence the outcome of synaptic competition and connectivity.

P1.4

Maternal dietary n-3 PUFAs deficiency alters microglia developmental activity in mice

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Polyunsaturated fatty acids (PUFAs) are essential fatty acids that mammals need to obtain through the diet. Imbalanced maternal dietary intake in PUFAs is an environmental risk factor for neurodevelopmental disorders such as autism, or schizophrenia and immune related diseases. N-3 PUFAs are key regulators of brain innate immune system processes. PUFAs also play a central role in brain development. From the last trimester of pregnancy until two years old in Humans, the brain accelerates its accretion of PUFAs, especially DHA. We previously demonstrated that depleting the diet in n-3 PUFAs from the first day of gestation alters some neuronal and microglial functions of the offspring.
Microglia, the brain innate immune cells, and the inflammatory mediators cytokines, are crucial actors of brain development from neurogenesis to cell migration, synapse formation and synaptic pruning. In particular, microglia are critical for neuronal wiring by eliminating immature and non functional dendritic spines during brain development. Such an effect is attributed to its phagocytic activity through activation of the classical complement cascade
However, until now, no direct evidence existed to support a connection between maternal n-3 PUFA intake, microglial activity and brain development.
In this study, we demonstrate that maternal n-3 PUFA deficiency alters developmental microglia-neuron interactions through activation of the complement system, leading to long-term behavioral impairment characteristic of neurodevelopmental disorders.

P1.5

Microglia-derived extracellular vesicles regulate the proliferation and differentiation of oligodendrocyte precursor cells

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Microglia have an enormous plasticity in their responses to CNS injury and can also display beneficial functions, playing an important role in fine-tuning inflammatory responses and promoting tissue repair *(Miron E et al., 2013 Nat Neurosci).* Despite the important role of microglia in various neurodegenerative disorders, the mode(s) of action of these cells in fostering or inhibiting CNS repair are far from being elucidated. Novel insights are needed in order to prevent the deleterious effects of inflammatory microglia and modulate them into a neurosupportive phenotype. Here, we investigated through what interactions and signals primary microglial cells orchestrate the endogenous reparative responses mediated by Oligodendrocyte Precursor Cells (OPCs), the glial cell type able to generate mature, myelinating oligodendrocytes *(Fumagalli M et al., 2011 Front Biosci).* We focused on extracellular vesicles (EVs) released from reactive microglia, which have been recently reported to play a crucial role in intercellular signalling between microglia and adjacent brain cells (i.e. neurons and astrocytes; *Prada I et al., 2013 Glia; Verderio C et al., 2012 Ann Neurol).* In detail, we explored whether EVs released from pro-inflammatory M1 or pro-regenerative M2 microglia can boost or block the proliferation and terminal differentiation of OPCs. Fluorescence microscopy analysis of OPCs exposed to EVs for 24h in the presence of the proliferative marker EdU showed that EVs produced by M1 cells inhibit OPC proliferation, while EVs released by M2 microglia increase OPC proliferation. Immunocytochemistry and western-blot analysis of markers of mature oligodendrocytes (e.g. CNPase and myelin basic protein MBP) revealed that 48h exposure to EVs derived from either M1 or M2 microglia, but not resting cells, promotes OPC maturation, with EVs derived from M2 cells displaying higher differentiation activity. Moreover, we also observed that a 10-day exposure to microglia-derived EVs favors myelin deposition in an *in vitro* system of OPCs co-cultured with DRG neurons. The possible role of microglia-derived EVs in modulating the OPC response is also suggested by preliminary data in an *in vivo* model of lysolecithin-induced corpus callosum demyelination.
Globally, these results unveil EVs as key players in microglia-OPCs cross-talk and suggest that the phenotype acquired by microglia greatly influences the proliferative/differentiation potential of OPCs.

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P1.6

Resolvins promote resolution of brain inflammation

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The brain innate immune system is mainly composed of microglial cells. Microglia are activated in response to an immune or inflammatory stimuli or a trauma, and then produce pro- and anti-inflammatory factors. These factors drive the innate immune response and can modulate neuronal activity and *in fine*, learning and memory. Although brain innate immune system defends brain tissue from aggression, chronic activation of microglia can also be deleterious. In the adult brain, chronic production of inflammatory cytokines can contribute to the pathogenesis of neurodegenerative diseases. Limiting the production of pro-inflammatory cytokines and enhancing the production of anti-inflammatory cytokines are crucial for neuron survival. New classes of small and local acting endogenous molecules have recently been identified. Specialized proresolving lipid mediators derived from n-3 polyunsaturated fatty acids (PUFAs), as the resolvins D1 and E1 (RvD1 and RvE1), are involved in the resolution of inflammation. However their involvement in the resolution of inflammation in microglial cells and the mechanisms by which they influence are unknown. Herein we studied the effects of resolvins on the resolution of inflammation in microglial cells stimulated with lipopolysaccharide. Our results indicated that resolvins were able to inhibit the production of pro-inflammatory cytokines and enhance the production of anti-inflammatory cytokines. Moreover, receptors of RvD1 and RvE1 were overexpressed during inflammation, reinforcing the idea that these molecules are involved in the resolution of inflammation. We also showed that resolvins promoted a phenotypic switch in microglial polarization toward a M2-like phenotype. These findings illustrate novel mechanisms through which PUFAs conferred anti-inflammatory and proresolving actions in inflamed brain.

P1.7

D-serine promotes NMDAR-mediated synapse formation and axonal refinement in the developing visual system of the Xenopus tadpole

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The N-methyl-D-aspartate receptor (NMDAR) has been shown to play a key role in synaptic plasticity. Unlike other neurotransmitter receptors, activation of the NMDAR requires the occupancy of both glutamate and glycine sites on the receptor. Recent studies have shown that the gliotransmitter D-serine binds the NMDAR glycine site with at least the same affinity as glycine and is an endogenous ligand for synaptic NMDARs in many brain areas. Here we examined the role D-serine plays in shaping neuronal activity and axonal remodeling in the developing visual system

of the Xenopus tadpole. We find acute D-serine (100 µM) wash-on enhances NMDAR mediated synaptic currents of optic tectal neurons, whereas degradation of D-serine by RgDAAO reduces NMDAR currents, indicating that D-serine is an endogenous NMDAR ligand and is normally present below saturating levels. To investigate the influence of D-serine availability on circuit maturation, we tested whether chronically elevating D-serine levels could influence the maturation of glutamatergic synapses. We find that tadpoles raised in D-serine (100 µM) for 2 days have higher frequencies of miniature excitatory postsynaptic AMPAR currents and higher retinotectal synaptic AMPA/NMDA ratios compared to control animals. Conversely, decreasing the amount of available D-serine, with a local injection of RgDAAO, results in a decrease in the amplitude and frequency of mEPSCs 24hrs after the injection. To examine the effects of D-serine on morphological development of retinotectal axons, images of EGFP expressing retinal axons were collected daily, over 4 days to assess growth and branch elaboration and at shorter (10 min) intervals to assess branch stabilization. We find that increasing available D-serine results in the hyperstabilization of retinal axon branches, with axonal arbors remaining less complex over 4 days of treatment with D-serine compared to control axons. To determine whether D-serine levels are modulated by glutamatergic neurotransmission we used D-serine sensitive amperometric biosensors and find that α-Amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid receptor (AMPAR) activation reliably results in an increase in D-serine release *in vivo*. These findings are consistent with the hypothesis that D-serine enhancement of NMDAR currents promotes synaptic maturation and leads to stabilization of axonal branches. Taken together, these results suggest that D-serine levels are modulated by glutamatergic neurotransmission *in vivo* and promote the maturation of retinotectal synapses and axonal stabilization.

P2.1

The dynamics of the tripartite synapse imaged live at the nanoscale using STED microscopy

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Astrocytes detect synaptic transmission and in turn regulate synaptic efficacy through glutamate uptake and gliotransmitter release. This bidirectional communication between astrocytes and neurons is thought to take place at the 'tripartite synapse', where astrocytic processes cover individual synapses, participating as the third partner together with pre- and postsynaptic compartments. Astrocytic coverage is potentially a critical parameter for tuning synaptic function,

by facilitating glutamate uptake and gliotransmitter action, while reducing the spill over of glutamate and gliotransmitters. However, beyond this general concept very little data exists on how astrocytic coverage influences synaptic transmission.
What we know so far about the structure of the tripartite synapse comes from snapshots of fixed samples captured by electron microscopy. This is because it is not possible to visualize the dynamic nature of astrocytic coverage (~100 nm) by conventional fluorescence microscopy, whose resolution is limited to ~250 nm.
To overcome this problem, we used a home-built STED microscope, which achieves high resolution imaging (~70 nm) in living organotypic brain slices in two colors. We visualized hyperfine astrocytic processes featuring highly branched and reticular structure. By co-labeling of spines, we could establish a measure of the degree of astrocytic coverage of dendritic spines. We found a large heterogeneity in astrocytic coverage, ranging widely between neighboring spines. Time-lapse STED imaging revealed that astrocytic coverage was by and large stable under unstimulated conditions over periods of about 1 hour.
By combining STED imaging with glutamate-uncaging and patch-clamp electrophysiology, we can now correlate in detail astrocytic coverage with functional parameters of synaptic transmission. We believe this approach will be the key to understanding how astrocytes regulate synaptic transmission at the level of single synapses.

P2.2

Modulation of Inap -mediated rythmic bursting by astrocytes network

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Glial cells can influence neuronal functions by multiple mechanisms including homeostasis of extracellular ions which have a direct impact on neurons firing pattern and their ability to discharge rhythmically. Rhythmic neuronal firing is an essential feature of neuronal circuits responsible for several vital repetitive movements like mastication, locomotion or respiration. Previous work from our laboratory has shown that astrocytes play a determinant role for rhythmogenesis in the trigeminal circuits responsible for mastication. In these circuits, neurons located in the dorsal part of the main sensory trigeminal nucleus (NVsnpr) fire rhythmically when the extracellular calcium concentration ($[Ca^{2+}]_e$) is lowered, and this rhythmic firing depends on a sodium persistent current (I_{NaP}) which is increased by the decrease of $[Ca^{2+}]_e$. Under physiological $[Ca^{2+}]_e$, intense stimuli like high frequency stimulation (40Hz) of sensory inputs to NVsnpr or local NMDA applications can reduce $[Ca^{2+}]_e$ and induce rhythmic bursting in parallel. Our recent work has shown that astrocytes play a central role in this effect probably by releasing S100β, a Ca^{2+}-binding protein, because their inactivation with intracellular dialysis of BAPTA or blockade of

endogenous S100β with an antibody prevent neuronal rhythmic bursting. However, disruption of bursting following BAPTA dialysis occurs only after allowing a long time (20-50 min) for BAPTA to diffuse to neighboring cells, suggesting that astrocytic coupling may be important for rhythmogenesis.
First, to insure that S100β acts by binding Ca^{2+} and lowering its extracellular concentration, we compared, in voltage clamp experiments, its effects to those of BAPTA on pharmacologically-isolated I_{NaP} current. Both BAPTA and of S100β increased the amplitude of I_{NaP} current and shifted its activation curve toward more hyperpolarized potentials. The anti-S100β antibody, which was previously found to abolish bursting, interfered with the ability of S100β to bind Ca^{2+}. The ability of S100β to alter neuronal firing was directly related to its effects on $[Ca^{2+}]_e$, and an S100β protein having two mutated residues in its Ca^{2+}-binding sites had no effect on neuronal firing and on $[Ca^{2+}]_e$.
To investigate the importance of astrocytic coupling for the induction of neuronal rhythmic bursting, we first tested if stimuli that induce rhythmic bursting in neurons (NMDA local application and high frequency stimulation of inputs to NVsnpr) increase dye-coupling in astrocytes. Astrocytic networks were evaluated by measuring the diffusion of biocytin to neighboring cells after patching a single astrocyte with an electrode containing it. Networks revealed under burst-inducing stimuli were about 3 times larger than those observed under control conditions (area of 85655 ± 15962 μm^2 and 29 ± 3 cells, n=13 vs area of 19374 ± 6973 μm^2 and 9 ± 3 cells, n=14; respectively). We then tested if coupling was important for the induction of rhythmic bursting in neurons and found that bath application of carbenoxolone (20 µM), a blocker of gap junctions, inhibited dye-coupling in astrocytes and disrupted NMDA-induced neuronal bursting in 9 of 11 cases tested. However, bursting could be restored by local application of S100β. These data indicate that astrocytes ability to form networks is important for the induction of neuronal rhythmic discharge potentially through a modulation of S100β release. This may have important functional implications regarding synchronisation of neuronal populations under normal and pathological conditions.

P2.3

Regional heterogeneity of rat brain astrocytes

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Astrocytes have been appreciated as active partners in the control of synaptic communication in the brain. There is a growing evidence supporting the regional functional differences in astrocytes regarding expression of transporters, enzymes, secreted molecules, glutamate uptake and

vulnerability to injuries. In the present study possible regional heterogeneity of astrocytes from rat cerebral cortex, hippocampus and brainstem was investigated combining RNA-Seq analysis, immunocytochemistry and lactate, aspartate and choline/acethylcholine (Ach) assays.
RNA-Seq analysis demonstrated regional heterogeneity of astrocyte specific markers. Brainstem astrocytes showed increased expression of GFAP (Glial Fibrillary Acidic Protein) compare to cortical astrocytes (2.48 fold) as well as increased expression of S100β (Calcium Binding Protein B) and AldoC (Aldolase C) in comparison to cortical and hippocampal astrocytes (2.66 and 2.02; 2.97 and 2.62 fold, respectively). While GS (Glutamine Synthetase) expression values were similar in cortical and hippocampal astrocytes and the lowest in brainstem (1.23 fold in comparison to cortical). RNA-Seq data were supported by immunocytochemistry. There was a statistically significant difference between fluorescence intensities in cultured astrocytes from cerebral cortex, hippocampus and brainstem as determined by one-way ANOVA. Moreover RNA-Seq analysis demonstrated different expression values of several transporters in three brain regions. Brainstem astrocytes showed increased expression of SLC44A1 (Choline transporter), SLC25A13 (Aspartate/Glutamate Carrier), SLC2A1 (Facilitated Glucose Transporter; GLUT-1) compare to cortical and hippocampal astrocytes (3.42 and 2.58; 3.02 and 3.14; 1.88 and 2.43 fold, respectively). However, in brainstem astrocytes expression levels of SLC1A2 (Glial High Affinity Glutamate Transporter; GLT-1) and SLC17A7 (Vesicular Glutamate Transporter; VGLUT1) were decreased in comparison to cortical and hippocampal astrocytes (1.53 and 1.67; 85.47 and 77.54 fold, respectively). Expression value of SLC16A1 (Monocarboxylic Acid Transporter 1) was the lowest in hippocampal astrocytes (2.14 fold). Hippocampal astrocytes demonstrated the tendency to increased expression of SLC9A1 (Sodium/Hydrogen Exchanger). In cultured astrocytes ionomycin induces calcium-dependent increase in extracellular release of lactate, aspartate and choline/Ach in astrocytes from all three regions – cortex, brainstem and hippocampus. Hippocampal astrocytes demonstrated increased lactate release compare to cortical (by 45%) and tendency to higher Asp and Ach release. Brainstem astrocytes exerted tendency to increased choline plus Ach release in comparison to both – cortical and hippocampal astrocytes.
The fact that hippocampal astrocytes exerted increased vulnerability to ionomycin-stimulated lactate release could support growing evidence indicating that lactate transport is essential for long-term memory formation in hippocampus. Ionomycin-induced increase in intracellular calcium level might lead to increased calcium-dependent and SLC9A19-mediated lactate release, while decreased expression of SLC16A1 in hippocampal astrocytes could contribute to higher levels of extracellular lactate. Altogether obtained data indicate on functional heterogeneity of astrocytes regarding the expression of specific markers GFAP and GS, as well as cell transporters and lactate release.

P2.4

EphB3 receptors regulate synaptic NMDAR functions

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Astrocytes are key partners of neurons and synapses. One of their main functions at CA3-CA1 hippocampal synapses is to regulate the activity of synaptic NMDA receptors (NMDARs) through the supply of the coagonist D-serine. Importantly, the detailed mechanism controlling the release of D-serine in the synaptic cleft is still unknown.
In this work, we have investigated whether astrocytic EphB3 receptors play a role in controlling NMDAR coagonist availability and thus NMDAR functions. Using electrophysiological approaches on acute hippocampal slices of adult mice, we here show that exogenous stimulation of EphB3 receptors with clustered ephrinB3-Fc ligands leads to an increase of NMDAR activity at CA3-CA1 synapses. Importantly, this modulation is due to an increase of the coagonist-binding site occupancy. Furthermore, disrupting endogenous ephrinB3-EphB3 interaction induces an impairment of synaptic NMDAR activity and its associated long-term synaptic potentiation. Both are rescued by exogenous supply of D-serine. We are presently carrying out experiments, to assess the role of astrocytic versus neuronal EphB3 receptors, in this pathway. All together, our data reveals that EphB3 receptors regulate synaptic NMDAR functions through the control of the coagonist-binding site occupancy.

P2.5

Astrocytes regulate heterogeneity of presynaptic strengths in hippocampal networks

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Dendrites are neuronal structures specialized for receiving and processing information through their many synaptic inputs. How input strengths are dynamically modified across dendrites in ways that are crucial for synaptic integration and plasticity largely remains unclear. We find that the presynaptic strengths of convergent inputs onto individual hippocampal pyramidal neurons are highly heterogeneous under basal conditions. This functional synapse heterogeneity is maintained by astrocyte Ca^{2+} signaling requiring NMDAR activation, astrocyte membrane depolarization and L-type Ca^{2+} channels; moreover, the mechanism is shared by the expression mechanism of heterosynaptic presynaptic plasticity that counterbalances input strengths. Intracellularly infusing NMDAR and Ca^{2+}-channel blockers into astrocytes or hyperpolarizing

astrocytes with archaerhodopsin (ArchT) rapidly drives homogenization of convergent presynaptic inputs. Our findings demonstrate the presence of an astrocyte-dependent cellular mechanism that enhances the heterogeneity of presynaptic strengths of convergent connections, which may help boost the computational power of dendrites.

P2.6

Astroglial CB1 receptors are required for *in vivo* hippocampal long-term potentiation of synaptic transmission

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Cannabinoids and endocannabinoids modulate synaptic function through the type-1 cannabinoid receptors (CB_1). In the hippocampus, the activation of neuronal CB_1 is known to modulate synaptic transmission and plasticity, and behavior. Interestingly, astrocytes also express functional CB_1 (astroglial CB_1) responsible for both memory impairments and a long-term depression (CB-LTD) of synaptic function induced by exogenous cannabinoids. Yet, whether the endogenous activation of astroglial CB_1 receptors can also modulate synaptic functions *in vivo* is not known.
Methods: *In vivo* recordings of evoked field Excitatory Postsynaptic Potentials (fEPSP) in the ipsilateral Schaffer Collateral-CA1 pathway were performed in anesthetized mice lacking CB_1 in astrocytes (GFAP-CB_1-KO) and wild-type littermate controls (GFAP-CB1-WT). Long-term potentiation (LTP) was induced by a high frequency stimulation (HFS) protocol (3 trains of 100Hz during 1s, 20s between each train). Pharmacological compounds, when used, were administered intraperitoneally (i.p.) before HFS.
Results: **1)** The deletion of CB_1 in astrocytes impairs the induction of LTP in the Shaffer collateral-CA1 synapses. Furthermore**, 2)** the administration of the selective non-competitive N-methyl-D-aspartate receptor (NMDAR) antagonist MK-801 (3.0 mg/kg) blocks the induction of LTP in WT mice. **3)** The administration of the NMDAR co-agonist D-Serine, two hours before HFS, restores the capacity of GFAP-CB_1-KO mice to express LTP.
Conclusions: These results reveal that astroglial CB_1 receptors are required for LTP in the hippocampus. Moreover, the administration of MK-801 confirms that this form of long-term plasticity is dependent on NMDAR transmission. Finally, the rescue effect of D-Serine suggests that

CB_1 in astrocytes regulates the availability of this molecule in the synapse hence controlling the induction of plasticity. Altogether these results demonstrate that the endocannabinoid system in astrocytes is important for normal synaptic function through the modulation of neuro-glial interactions.

P2.7

Astroglial type-1 cannabinoid receptors (CB_1) are necessary for long-term object recognition memory

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Astrocytes express a wide variety of G protein-coupled receptors (GPCR) that can influence cognitive functions such as learning and memory. Cannabinoids and endocannabinoids modulate memory processes through the GPCR, CB_1 receptor. Interestingly, similarly to neurons, astrocytes express functional CB_1 receptors capable of modulating the effects of exogenously administered cannabinoid agonists on hippocampal synaptic plasticity and working memory. However, the endogenous roles of astroglial CB_1 receptor in long-term memory remain unknown.
To assess the role of astroglial CB_1 receptor in long-term memory, we used conditional mutant mice lacking CB1R specifically in astrocytes (GFAP-CB_1R-KO), drug applications (intra-peritoneal and intra-dorso hippocampal) and tested memory performances through an object recognition task performed in a L-maze. GFAP-CB_1-KO mice have impaired long-term object recognition memory. Notably, administration of D-serine, a gliotransmitter that is a co-agonist at the N-methyl-D-Aspartate receptor (NMDAR), restores the long-term memory deficit when injected systemically. In line with this idea, rising endogenous levels of D-serine, through the inhibition of its degrading enzyme DAAO, also rescued the performances of GFAP-CB_1R-KO animals. Interestingly, local infusion of D-serine into to dorsal hippocampus also completely restores long-term memory deficits.
Then, hippocampal astroglial CB_1R are necessary to guarantee appropriate long-term memory through the modulation of the co-agonist site of the NMDAR. This study provides the first proof of a physiological role of astroglial CB_1R in behavior and reveals an unexpected role for astroglial CB1 receptors in memory formation.

P2.8

Hippocampal LTP depends on astrocytic IP_3 receptors

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Astrocytic Ca^{2+} signaling is required for LTP at the Hippocampal Schaffer Collateral to CA1 (SC-CA1) synapses. Controversy, however, remains as IP_3 receptor (IP_3R) type-2 knockout mice (IP_3R-2KO), which are reportedly deficient in astrocytic Ca^{2+} signaling, exhibit normal LTP. It is possible that an astrocytic Ca^{2+} channel hitherto unknown is required for LTP and that some Ca^{2+} transients still prevail locally, at the level of the processes, in IP_3R-2KO mice. In the current study, we address this hypothesis by visualizing sub-cellular Ca^{2+} dynamics within astrocytic processes, focusing on Ca^{2+} release through all IP_3R subtypes (1,2,3) and their role in LTP.

To image Ca^{2+} dynamics in astrocytic processes we used two-photon imaging of GCaMP3 expressed in cultured hippocampal slices. We also performed two-photon Ca^{2+} imaging in acute slices, in this case we loaded the Ca^{2+} indicator Fluo-4 via an astrocytic whole-cell patch-pipette. To evoke Ca^{2+} responses in astrocytes, we bath applied the group 1 metabotropic glutamate receptor agonist DHPG, or performed high frequency stimulation (HFS, 1sec at 100Hz) of the Schaffer Collaterals. In contrast to the premise of previous studies, we observed substantial astrocytic Ca^{2+} responses in slices prepared from IP_3R-2KO mice. As the activation of astrocytic group 1 mGluRs is known to trigger Ca^{2+} release via IP_3Rs, we decided to investigate the contribution of other IP_3R subtypes. To this end we repeated the above experiments using hippocampal slices prepared from IP_3R type-2/-3 double KO mice (IP_3R-2/3KO) and used heparin, introduced via an astrocytic whole-cell patch-pipette, to inhibit all IP_3R subtypes. We thus identified two new astrocytic Ca^{2+} channels, namely IP_3R-1 and IP_3R-3.

Having identified two new functional Ca^{2+} channels in astrocytic processes we tested their involvement in LTP. In accordance with Ca^{2+} imaging data, 2xHFS-LTP was intact in slices prepared from IP_3R-2KO, and IP_3R-2/3KO mice. Finally, inhibiting all astrocytic IP_3R subtypes with heparin inhibited 2xHFS-LTP. Our results show for the first time that IP_3R-1 and IP_3R-3 are functional Ca^{2+} channels within astrocytes, and that they could be required for LTP induction at hippocampal CA3-CA1 synapses.

P2.9

Two-photon STED imaging of synapses and their glial partners *in vivo*

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The traditional view of glial cells as only playing a role in nurturing and protecting neurons has proven to be too narrow, as mounting evidence indicates that astrocytes and microglia actively contribute to several important aspects of synapse development and function. Hence, detailed knowledge of how these glial partners communicate with neurons, and synapses in particular, is key to a more comprehensive understanding of brain physiology. However, very little is known about how glia cells dynamically interact with synapses in the living brain to coordinate its function.

Progress in this regard has been impeded by the inability of existing intravital imaging approaches like two-photon microscopy, to properly visualize the fine structural details and dynamics of synapses and glial cells in living brain tissue.

Therefore, we adapted super-resolution STED microscopy to image neural and glial morphology in the intact mouse brain *in vivo* with greatly improved spatial resolution. The *in vivo* approach is necessary for preserving neuro-vascular coupling of astrocytes and helps to keep microglia in their normal non-activated state. Our home-built STED microscope combines two-photon excitation with pulsed STED quenching, which maximizes the gain in spatial resolution. We used a silicon-oil objective to reduce spherical aberrations from the mismatch in refractive index between the optics and brain tissue. Neurons (and their dendrites) were labeled with YFP and microglia with GFP using transgenic animals. We are still working on the development of bright constructs for AAV-viruses to have a robust labeling strategy for the astrocytes. The YFP and GFP fluorescence signals are effectively separated by spectral detection and linear unmixing.

Here, we show the development of our labeling strategy for astrocytes, and we demonstrate the feasibility and power of our super-resolution approach by time lapse imaging of glial cells and synapses in layer 1 of neocortex in anesthetized mice.

P3.1

Altered morphological dynamics of activated microglia after induction of *status epilepticus*

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The morphology of microglial cells is characterized by numerous fine processes, which are strikingly dynamic. In the normal brain, they constantly scan the surrounding brain tissue and rapidly move towards sites of acute injury or danger signals. These microglial dynamics are thought to be critical for brain homeostasis. Under pathological conditions microglial cells undergo 'activation', which modifies many of their molecular and morphological properties. Investigations on the effects of activation on motility are limited and have given heterogeneous results. In particular, little is known about how microglial motility is altered in epilepsy, which is associated with a strong inflammatory reaction and microglial activation.
We used a mouse model of *status epilepticus* induced by kainate injections and time-lapse two-photon microscopy to image GFP-labelled microglia in acute hippocampal slices. We studied how microglial activation affected the motility of microglial processes, including basal motility and their responses to local triggering stimuli. Our study reveals that microglial motility was largely preserved in kainate-treated animals. In addition, whereas the velocities of microglial processes during basal scanning and towards a laser lesion were unaltered 48 hours after *status epilepticus*, we observed an increase in the size of the territory scanned by single microglial processes during basal motility and an elevated directional velocity towards a pipette containing a purinergic agonist.
In conclusion, microglial activation differentially impacted the dynamic behaviors of microglia in response to specific acute noxious stimuli, which may be an important feature of altered microglial behavior during pathophysiological conditions.

P3.2

Roles of reactive astrocytes in Alzheimer's disease?

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Astrocytes have important roles in the brain. In neurodegenerative diseases, such as Alzheimer's disease (AD), astrocytes become reactive. Astrocyte reactivity is essentially characterized by morphological changes and over-expression of intermediate filament proteins. But how the normal supportive functions of astrocytes are changed by their reactive state is unclear. Any alteration in theses functions could have important consequences on neuronal activity and viability. In this study, we aim to understand the contribution of reactive astrocytes to neuronal dysfunction in the context of AD.
We reported recently that the JAK2/STAT3 pathway (Janus Kinase 2/ Signal Transducer and Activator of Transcription 3) is responsible for astrocyte reactivity in several models of ND (Ben Haim et al., 2015). Here, we used adeno-associated viral vectors (AAV) to modulate this pathway specifically in astrocytes in two mouse models of AD (APP/PS1dE9 and 3xTg-AD mice).
Hippocampal injection of an AAV encoding SOCS3 (Suppressor Of Cytokine Signaling 3), the endogenous inhibitor of the pathway, de-activated astrocytes in both mouse models of AD. We showed by immunohistochemistry and biochemistry, that amyloid load was decreased and that the concentration of soluble amyloid-beta tended to be reduced in APP/PS1dE9 mice injected with AAV-SOCS3. Recruitment of microglial cells at amyloid plaques was also significantly decreased by SOCS3. Expression of synaptic markers was not changed by SOCS3. Finally, we evaluated the effects of reactive astrocytes on anxiety and memory in 3xTg-AD mice with the elevated plus maze and the Y maze. We found that anxiety tended to be reduced by AAV-SOCS3 but memory was not restored. These results suggest that reactive astrocytes enhance several alterations linked to AD pathology.
This original and selective approach to modulate astrocyte reactivity in the mouse brain makes it possible to directly assess the contribution of reactive astrocytes to AD.

P3.3

Membrane dynamics of AQP4: a new key pathway for physiopathological brain cell communication?

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Neuro-inflammation is a pathological hallmark of most brain disorders and is known to involve AQP4, a specific water channel expressed on the astrocytes, to modulate the edematous component associated with inflammation. In this project, we aim to address a potential additional role of AQP4 in modulating the synaptic activity. Considering that regulation in key brain

compartment such as the area around synapses need to be conceptually revisited to include transporter diffusion (1), we first explored whether AQP4 is diffusing along the astrocyte membrane. To tackle this issue, we used a combination of cutting-edge high-resolution imaging approaches to track single AQP4 in live cells (quantum dot imaging). We showed for the first time, that in culture the motility of AQP4 increases in presence of neurones compared to pure astrocytes (0.07 µm²/s). We then explored whether the dynamic of AQP4 is implicated in the glutamatergic synapse physiology which could involve the modulation of the volume of the synaptic cleft and we saw that modifications on the synaptic activity (incubation with glutamate or TTX) affect AQP4 motility in opposite way: with glutamate we found a persistent decrease of diffusion (~-50%) while with TTX we saw an increase in the mobility (~+70%). Finally, we are currently testing whether AQP4 diffusion could be disturbed during brain inflammation participating to the pathophysiology. The role of AQP4 in pathology is mainly investigated by exploring the effects of auto-antibodies against AQP4 from neuromyelitis optica (NMO) patients, a specific inflammatory disorder of the central nervous system (2). We believe this condition could be the archetype of specific AQP4 dysfunction that could help to understand mechanisms probably more ubiquitously disturbed. Preliminary data show that the auto-antibodies have an impact on motility of only one AQP4 isoform. Altogether it could position AQP4 dynamic as a central mechanism whose early deregulation could lead to glutamate excitotoxicity in inflammatory conditions. These new mechanisms related to membrane diffusion could be ubiquitously disturbed in a wide range of inflammatory disorders and their understanding could pave the way toward new therapeutic strategies.

(1) Murphy-Royal et al., 2015. ***Surface diffusion of astrocytic glutamate transporters shapes synaptic transmission.*** *Nat Neurosci. 2015 Feb;18(2):219-26. doi: 10.1038/nn.3901.*
(2) Pittock SJ, Lucchinetti CF. ***Neuromyelitis optica and the evolving spectrum of autoimmune aquaporin-4 channelopathies: a decade later.*** *Ann N Y Acad Sci. 2015.*

P3.4

Effect of an omega-3/antioxidants supplemented diet on cognitive alterations and neuroinflammatory processes associated with obesity

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Obesity is a metabolic and inflammatory disorder that represents a major risk factor for development of comorbidities such as cardiovascular diseases or diabetes. It is also associated with an increased prevalence of cognitive dysfunctions that represent important risk factors for

aggravation of obesity and related outcomes. Reducing the development of such alterations in the context of obesity may be therefore a way to improve health and quality of life of obese subjects. Converging clinical and experimental studies have suggested that inflammatory processes, which are often associated with severe obesity, might contribute to the development of cognitive alterations, in particular when they occur in brain areas associated with learning and memory such as the hippocampus. Previous studies from our laboratory have suggested that cognitive alterations observed in inflammatory conditions may be in part mediated by changes in synaptic plasticity. Interestingly, nutriments such as n-3 polyunsaturated fatty acids (n-3 PUFAs) or antioxidants (AO) have been shown to protect against the development of cognitive alterations in different inflammatory conditions and are potent modulators of neuroinflammation. However, their effect in the context of obesity still remains relatively unknown. Thus, the aim of this study was to evaluate: 1) if a diet supplemented with n-3 PUFAs and AO improved cognitive deficits displayed by a genetic mouse model of obesity (db/db mice), 2) if this potential cognitive improvement was associated with reduced inflammation and/or 3) with changes in synaptic plasticity. Chronic consumption (12 weeks) of the supplemented diet reversed hippocampus-dependent spatial memory deficits displayed by un-supplemented db/db mice in a water maze task. This supplemented diet increased circulating levels and hippocampal expression of the anti-inflammatory cytokine interleukin-10. However, it did not improve the activation of intracellular signaling pathways associated with inflammation. Regarding synaptic plasticity, this enriched diet restored protein levels of GluA2 subunit of the AMPA receptor in the hippocampus of db/db mice and decreases protein expression of GluN2A and GluN2B subunits of the NMDA receptor in both genotypes, suggesting that the PUFAs and AO enriched diet may change synaptic plasticity. Electrophysiological experiments are now in progress to assess the effect of the supplemented diet on hippocampal long-term potentiation and long-term depression involved in cognitive performances. These findings should provide valuable data for introducing new nutritional strategies for the improvement of cognitive alterations associated with obesity by targeting synaptic plasticity.

P3.5

Specific alteration of NMDA receptor synaptic organization and trafficking in autoimmune psychosis

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The link between schizophrenia and immune system dysregulations is an old concept that regained strong support over the last years. Recently, the presence of circulating autoantibodies against the N-Methyl-D-Aspartate receptor (NMDAR) has been reported in schizophrenic patients, suggesting a potential role of such antibodies in the emergence of psychotic disorders. However, the cellular and molecular mechanisms involved by these molecules remain unexplored.
By using a combination of high resolution imaging and immunocytochemistry, we tested the impact of NMDAR autoantibodies (NMDAR Ab) from schizophrenic and healthy individuals on NMDAR surface trafficking. Interestingly, we observed a differential effect between NMDAR Ab of different origins, highlighting the pathogenicity of autoantibodies specifically produced by schizophrenic patients. Unlike NMDAR Ab from healthy subjects, the presence of antibodies from psychotic patients rapidly disturbs the surface trafficking of NMDAR, leading to a complete disruption of their synaptic distribution. These data suggest the involvement of a NMDAR "synaptic emptying" phenomenon, possibly caused by a defective synaptic anchorage. In order to understand whether a loss of NMDAR synaptic retention is part of this pathological process, we then studied the impact of NMDAR Ab on the EphrinB2 receptor (EphB2R), a major and direct membrane partner of the NMDAR. Once again, we showed that only the presence of antibodies from schizophrenic patients affects the EphB2R surface trafficking, which could critically impact on NMDAR synaptic retention.
Together, these data provide the first evidence that circulating NMDAR Ab exhibit different capacities to disorganize NMDAR molecular complexes in excitatory synapses, opening new avenues of research to counteract NMDAR alterations in schizophrenia.

P3.6

Single nanoparticle tracking of receptors in psychotic disorders

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The link between psychotic disorders and immune system dysregulations is a well-established concept that has regained strong support over the last decade's and, recently a subgroup of schizophrenic (62%) and bipolar patients (56%) were found to be positive for an envelope (Env)-protein produced from a human endogenous retrovirus (HERV)-W. Normally, the expression of this protein is restricted by epigenetic and posttranslational processes however, active transcripts are especially found in patients with neuroinflammatory related neuropsychiatric diseases.
One hypothesis aiming to explain the underlying mechanisms of schizophrenia and psychotic disorders is the glutamate-hypofunction hypothesis which has evolved from the fact that N-Methyl-D-Aspartate receptors (NMDAR) antagonists produce symptoms that mimic psychosis. This hypothesis could potentially explain several symptom dimensions of schizophrenia and psychotic related disorders including cognitive dysfunctions related to the hippocampus. Furthermore, recent work concerning surface diffusion of NMDARs in hippocampal neurons reveals evidence of a direct link between receptor dynamics and LTP induction in developing neurons, leaving any perturbation of receptor-diffusion properties linked to profound implications on neuronal activity and memory formation.
We use a combination of single nanoparticule (Quantum Dot) tracking and classical immunohistochemical imaging in hippocampal neurons as a first approach to explore the impact of the Env-protein on membrane NMDAR surface trafficking and the disturbance of NMDA receptor-mediated signaling under psychotic related conditions.
We have observed that the Env-protein has the potency to affect surface dynamics of the NMDA receptor in a subunit specific way, specifically in the neuronal postsynaptic area. Glial cells were necessary for this process to occur, and this was furthermore accompanied by an increased NMDAR surface expression.
Together, these imaging data provide evidence that the Env-protein, produced from a human endogenous retrovirus, influence the synaptic NMDA receptors by means of neuron-glial reactivity.

P3.7

Laforin/malin E3-ubiquitin ligase complex is implicated in the regulation of the subcellular localization of GLT-1 (EAAT2) glutamate transporter

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Lafora disease (LD) is a fatal autosomal neurological disorder caused by mutations in *EPM2A,* encoding the dual-specificity phosphatase laforin, or *EPM2B,* encoding the E3 ubiquitin ligase malin. LD is characterized by neurodegeneration, epilepsy and the accumulation of insoluble and poorly-branched polyglucosans, called Lafora bodies, in brain and other peripheral tissues. Recent years have witnessed progress with respect to our understanding of the disease. However, the molecular basis underlying the epilepsy are still poorly known and mainly focused on neuronal dysfunction.
Astrocytes are the most abundant cells in the CNS that provide nutrients, recycle neurotransmitters, as well as fulfill a wide range of other homeostasis maintaining functions. In the last two decades, the relevance of astrocytes in different neurological disorders, including epilepsy, has been repeatedly demonstrated.
One of the main roles of astrocytes is removing glutamate from the synaptic space and it is carried out by several glutamate transporters. EAAT2 is the major responsible of glutamate uptake in the CNS and its impairment is associated to hyperexcitability and neuronal death. The presence of EAAT2 at the plasma membrane is regulated by the surface-ubiquitin mediated turnover of the transporter.
In this study, we observed that glutamate uptake is impaired in primary astrocytes from Epm2a-/- and Epm2b-/- mice compared to control mice. We also observed that EAAT2 is decreased at the membrane of these astrocytes and increased in neuroblastoma cells overexpressing laforin and malin and those changes correlate with changes in the ubiquitination levels of EAAT2. All these results suggest a disfunction of EAAT2 in LD that could be contributing to the epilepsy of the disease.

P3.8

Involvement of P2X4 receptor in Alzheimer disease

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Alzheimer disease (AD) is a neurodegenerative disease affecting more 35 million people worldwide and costs about 600 billion dollars. Despite years of research, the mechanisms leading to this pathology are still debated. Studies have shown the importance of inflammation in AD progression. Microglia, the immune cells of the brain, are a key players during the inflammatory response particularly trough cytokines and pro-inflammatory mediators release. Yet, microglia have both protective and detrimental functions in AD. The ATP-activated ion channels P2X4 receptors (P2RX4) are expressed de novo in activated microglia where they control the release of BDNF or PGE2 and sustain network excitability. Genetic ablation of P2X4 receptors results in the attenuation of phenotypes associated with several chronic central nervous system disorders such as neuropathic pain, spinal cord injury or epilepsy. Despite these observations, the involvement of

P2RX4 in chronic neurodegenerative disorders has not been investigated. The aim of this work is to understand the role of P2RX4 during AD. To answer this question we crossed P2RX4-deficient mice and APP/PS1 strain, a well characterized mouse models of AD. We evaluated the disease progression at 9 and 12 months through behavioral, immune-histochemical and biochemical analyses.
Using Thioflavine staining, amyloid plaques deposit was scored in each genotype. Compared to APP/PS1 mice, the number and size of amyloid plaques are increased in APP/PS1 P2X4$^{-/-}$ mice at 9 months. These data correlate with preliminary behavioral analysis showing a greater impairment of spatial memory in female APP/PS1 P2X4$^{-/-}$. Using immunostaining, P2X4 receptors were localized predominantly in activated microglia surrounding amyloid plaques suggesting that P2RX4 could regulate phagocytosis. However, we found no evidence for an impaired phagocytosis of Aß peptide by P2RX4-deficient primary microglia.
Our results suggest that microglial P2X4 receptors by controlling specific functions of activated microglia participate to Aß deposit clearance during AD progression. Further work is necessary to understand the cellular mechanisms linking P2RX4activation to specific microglial functions.

P3.9

Microglial activation causes selective dentate gyrus disruption and memory impairment in experimental multiple sclerosis

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The substrate of early memory impairment in multiple sclerosis is poorly understood and it remains unknown whether some hippocampal regions could be more vulnerable than others. We first demonstrated that hippocampal-dependent memory was impaired at the early stage of experimental autoimmune encephalomyelitis (EAE), a mouse model of multiple sclerosis. Then, using in vivo diffusion-tensor imaging (DTI) to screen for regional vulnerability within the hippocampus, we found a selective decrease of fractional anisotropy in the molecular layer of the dentate gyrus of EAE-mice. Morphometric analyses confirmed the occurrence of a selective neurodegenerative process in this structure. Glutamatergic synaptic transmission and long-term

synaptic potentiation were also selectively impaired in the dentate gyrus but not in CA1. Microglia was activated in every hippocampal regions and treating mice with the microglial inhibitor minocycline prevented DTI, morphological, electrophysiological and behavioral impairments. These results are the first to link early memory impairment in multiple sclerosis to disruption of the dentate gyrus by demonstrating that dentate gyrus structure and function are more vulnerable to the neurotoxic effect of microglial activation than other hippocampal regions. By capturing some of these features non-invasively with DTI, our work paves the way toward rapid translation to humans.

P3.10

Astrocytic phenotype changes after early mild traumatic brain injury

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Background: Traumatic brain injury (TBI) is the leading cause of death and disability in the pediatric population. Moreover, mild TBI (mTBI) is frequently under-diagnosed, with patients being rapidly discharged from hospital when no hemorrhages or substantial behavioral deficits are observed during the acute phase. However, growing clinical evidence shows that patients with mTBI endure behavioral impairments for months or years after the initial injury. So far there is no treatment and little is known on the molecular and cellular mechanisms behind short and longterm behavioral changes.
Astrogliosis is observed alongside behavioral deficits in several types of brain damage, and the correct functioning of astrocytes, located at the interface of vascular and neuronal structures, is essential for brain repair. Our team therefore hypothesizes that molecular transformation of the astrocyte phenotype is involved in the progression of injury and cognitive impairment after mild juvenile TBI.
Methods/Results: To tackle this important question, we developed a new rodent model of mTBI inducing a closed-head injury using an electromagnetic impactor mounted with a 3 mm tip piston on pnd 17-old mice. We compared the outcomes of mild and moderate TBI (1 mm impact depth, 2 m/s speed; 3 mm impact depth, 3 m/s speed respectively) with sham mice.
Increasing TBI severity caused a significant increase in the time to resume exploratory behavior after the injury (101,9 % and 162,5 % increase compared to sham, in mild and moderate respectively). Subdural and intraparenchymal bleeding was observed after perfusion in 61% of the mice from the moderate group, whereas no visible bleeding was found in mild and sham animals. Mild TBI did not cause significant neuronal death. However, both grades of TBI induced significant

blood brain barrier dysfunction in the ipsilateral corpus callosum 3 days after the injury (IgG infiltration; 17,9 % and 73,5 % increase compared to contralateral, in mild and moderate respectively), which was not significant in sham. GFAP and AQP4 staining were increased even in the milder TBI group, showing astrocytic activation. The role of AQP4 has largely been discussed in relation to edema formation, but AQP4 has also been proposed to be involved in ATP release and consequent activation of the purinergic receptor P2, including P2X4 receptors (P2X4R). We found increased P2X4R levels after mTBI and thus hypothesized that this could affect astrocytic Ca^{2+} signaling. Indeed, astrocytes from mTBI *ex vivo* brain slices had increased Ca^{2+} responses which reverted to normal in P2X4R knock out mice. Preliminary observations of mice behavior suggest that mTBI could be promoting anxiety (elevated plus maze test) and cognitive decline (novel object recognition test) up to 1 month after the injury.
Conclusions: Astrocytic activation seems to be a prominent feature of the neuroinflammatory response after juvenile TBI, and changes in astrocytic phenotype could be altering not only water movement but also vascular and neuronal responses. In fact, it has recently been shown that astrocytic Ca^{2+} changes are key modulators of vascular autoregulation, and thus post-TBI vascular dysfunction could be happening through this pathway. Follow-up of injured juvenile mice into mature/old age will give us an insight of the impact of early-life TBI in astrocytic phenotype changes through lifespan. Comparison of our results with models of adult TBI and with molecular observations in clinical samples from collaborating teams will hopefully provide the basis for better diagnosis and early treatments to prevent the long-lasting consequences of TBI.

P3.11

Non – cell autonomous neuroprotective effect of carbon monoxide: microglia – to – neuron communication

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Apart from being one of the leading causes of morbidity and mortality worldwide, brain ischemia is also a main cause of disability, leaving a great percentage of survivors dependent on others for simple activities. A major portion of the brain damage that slowly ensues following the ischemic event is caused by unrestrained inflammatory response. The main cells responsible for orchestrating such a response are microglia, the 'resident immune cells in the brain'. Microglia react to a wide array of stimulus, including ischemia, by overexpressing and releasing inflammatory factors that not only potentiate its own phagocytic capacity, but also promote the recruitment of peripheral immune cells. Overall, neuroinflammation is protective to the central

nervous system, but exacerbated microglial activities can lead to disastrous consequences, since many secreted molecules, like TNF-α and ROS are potentially cytotoxic, and contribute to a neurotoxic environment responsible for propagating damage. Developing strategies for modulating microglial activity is a major concern nowadays, which is not limited to therapeutic solutions for brain stroke, but also for neurodegenerative diseases like Alzheimer's or autoimmune diseases, such as multiple sclerosis.
The endogenous molecule carbon monoxide (CO) has been shown to possess anti-neuroinflammatory properties using *in vitro* and *in vivo* approaches, including microglial cell culture models and rat models of hemorrhagic stroke. Thus, our objective was to study CO as modulator of microglial activity, in particular in what concerns their communication with neurons, by promoting neuronal viability and limiting inflammatory output of LPS-activated microglial cells.
BV2 microglia and SH-SY5Y neurons were used as cell models for assessing cell-to-cell communication. CO-releasing molecule A1 (CORM-A1), a boranocarbonate compound that liberates CO spontaneously, was used to deliver CO to cells. We found that, in accordance with the literature, CORM-A1 pre-treated BV2 cells limited the inflammatory response. Furthermore, conditioned medium from activated microglia with LPS promoted SH-SY5Y mortality when challenged with a pro-oxidant (*tert*-butyl-hydroperoxide). However, when LPS-activated microglia were pre-treated with CORM-A1 there was a decrease in neuronal cell death. The pattern of BV2 cytokine secretion was also analyzed in the presence and absence of CORM-A1 treatment.
In conclusion, CO modulates microglia and neuron communication and limits neuroinflammation. This work reinforces the idea that CO might be used in the future as a viable therapeutic molecule to alleviate the inflammatory response. This work will provide basis for trying to unveil the molecular mechanisms involved in CO-mediated protection in the context of neuro-inflammation.

P3.12

Remodeling of glial coverage of glutamatergic synapses in the rat nucleus tractus solitarii after ozone inhalation

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Besides the well described inflammatory and dysfunction effects on the respiratory tract, accumulating evidences indicate that ozone (O_3) exposure also affects central nervous system functions. However, the mechanisms through which O_3 exerts toxic effects on the brain remain poorly understood. We previously showed that O_3 exposure caused a neuronal activation in regions of the rat nucleus tractus solitarii (NTS) overlapping terminal fields of vagal lung afferents. Knowing that O_3 exposure can impact astrocytic protein expression, we decided to investigate whether it may induce astroglial cellular alterations in the NTS.
Using electron microscopy and immunoblot techniques, we showed that in O_3-exposed animals, the astrocytic coverage of NTS glutamatergic synapses was 19 % increased while the astrocyte

volume fraction and membrane density were not modified. Moreover, the expression of GFAP (glial fibrillary acidic protein) and S100b, which are known to be increased in reactive astroglia, did not change. These results indicate that O_3 inhalation induces a glial plasticity that is restricted to the peri-synaptic coverage without overall astroglial activation. Taken together with our previous observations, they support the conclusion that O_3-induced pulmonary inflammation results in a specific activation of vagal lung afferents rather than non-specific overall brain alterations mediated by blood-borne agents.

P3.13

D-serine rescues early synaptic plasticity deficits in 3xTg mice

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At early stages of Alzheimer's disease (AD), intracellular accumulation of Aβ leads to cognitive and synaptic transmission impairment. Such deficits are reproduced in the triple transgenic model 3xTG mice in which basal excitatory synaptic transmission and long-term potentiation (LTP) in the hippocampus are significantly impaired long before plaque deposits. LTP induction at CA3-CA1 synapses depends on NMDA receptors (NMDARs), and we have shown previously that these receptors were gated by glial D-serine. We here investigated whether the synaptic plasticity deficits observed in 3xTg mice at early age could be due to a reduced level of occupancy of NMDAR glycine-binding sites. To this end we recorded field excitatory postsynaptic potentials (fEPSPs) at CA3-CA1 synapses in acute hippocampal slices obtained from 6-7 months old mice. As previously described, both input/output relationship and LTP were impaired significantly. We then assessed the level of occupancy of NMDAR co-agonist binding site by applying exogenous D-serine in the bath. The increase in NMDAR-mediated fEPSPs induced by D-serine was larger in 3xTg mice, indicating that the level of occupancy of NMDAR co-agonist site is lower in these animals. Most importantly, exogenous D-serine rescued completely LTP and LTD induction in the triple transgenic mice. Altogether, these results suggest that early deficits in AD are associated with a decrease in D-serine in the synaptic cleft.

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General Information

Meeting venue

The symposium will take place at the Institut d'Optique d'Aquitaine of Talence, located on the University of Bordeaux campus, southwest of the famous city of Bordeaux.

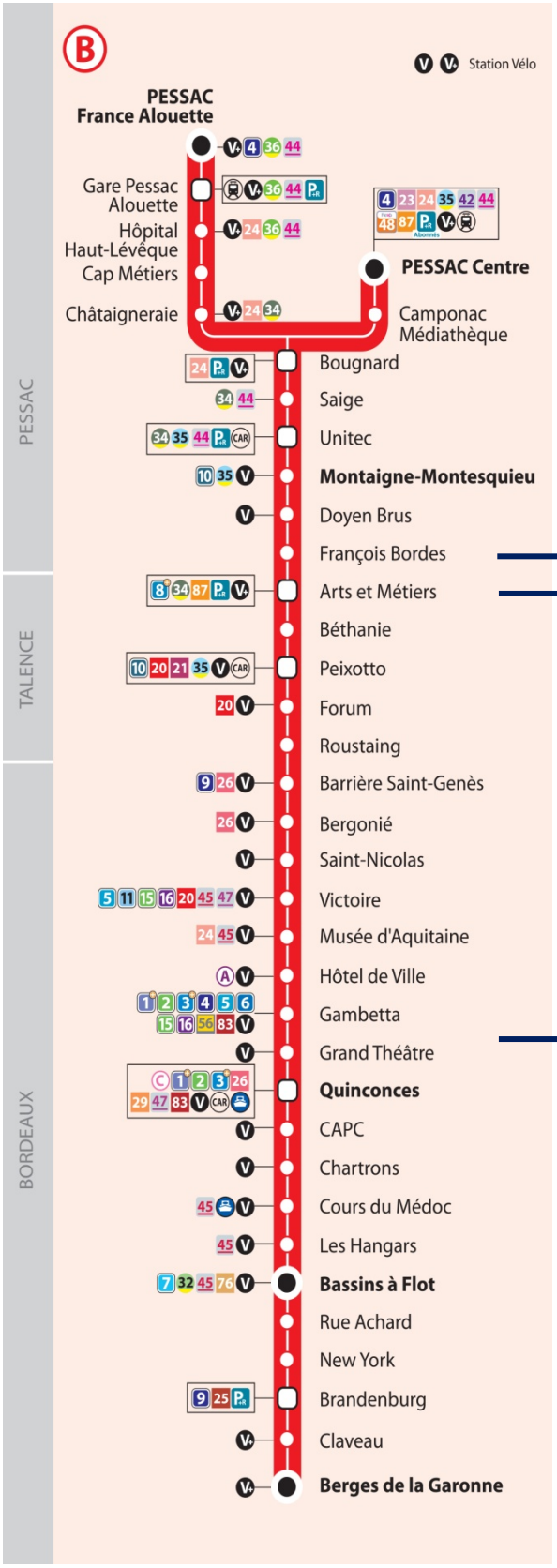
GPS: Latitude 44.804258 - Longitude -0.60455 *(Access IOA)*

Address: Rue François Mitterrand (Allée René Laroumagne)
33400 Talence Cedex
Tel. 05 40 00 69 36

How to get there:
Tram: B line – *Arts-et-Métiers* station from Bordeaux or *François Bordes* station from Pessac (recommended)

… and then a short 2 min walk uphill!!! (see next page for the map)

TBC Transportation Map (Tram, B Line)



2 stations near the IOA

François Bordes
Arts-et-Métiers

Best Western hôtel
Grand Théâtre station

SOCIAL EVENT: GALA DINNER
Thursday, October 1st

Château Carbonnieux is a *Grand Cru Classé* (Classified Growth) of the Graves appellation for both its red and white wines. It is located at Léognan, 20 minutes from Bordeaux. Château Carbonnieux was founded in the 13th century by the Benedictine monks of Sainte-Croix Abbey, it is one of the oldest estates in the Bordeaux region.

CHÂTEAU CARBONNIEUX
PESSAC-LEOGNAN
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Wifi access at the IOA

A limited number of logins will be made available by the IOA staff and handed at Registration.

The REAUMUR and EDUROAM networks are also available.

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Agnès NADJAR	NutriNeurO *Maître de conférences*
Aude PANATIER	NeuroCentre Magendie, NCM *Chargée de recherche CNRS*