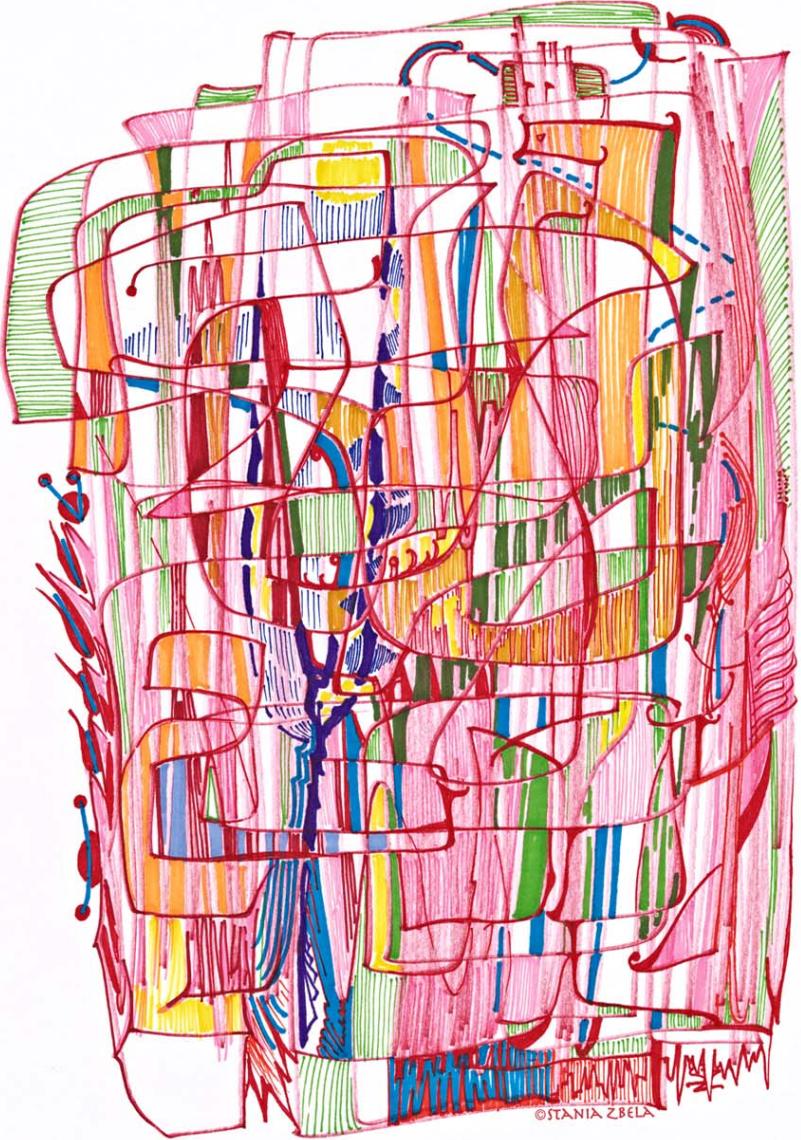


Evolution of the Brain

DNF symposium - 13.05.16



3rd DNF Symposium: Evolution of the Brain

Organisation

Main organizers: *Egbert Welker & Dirk Fasshauer*

Organizing committee: *Claudia Bagni*

Logistics and Printing: *Eric Bernardi*

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Image on the front side: *Art work by Stania Zbela entitled "Evolution of the Brain"*

3rd DNF Symposium: Evolution of the Brain

Since 2014, the Department of Fundamental Neurosciences (DNF) organizes an annual one-day symposium on different topics of neurosciences with the aim to present the latest developments from the different research groups working at the DNF. Our department hosts 15 research laboratories gathering a total of 130 scientists and staff members.

The DNF symposium combines lectures by international speakers, by members of the DNF and the Lemanic Neuroscience Community, and by speakers selected from submitted abstracts. The talks are interleaved with a dynamic poster sessions and we wish to provide a pleasant milieu for stimulating discussions during the entire day and more informally during the social event at the DNF that concludes the symposium.

This year the organizers, Dirk Fasshauer and Egbert Welker, propose an exciting program highlighting aspects of brain evolution – the speakers will discuss how the first synapses in simple animals are established and how synaptic plasticity is regulated in a more complex vertebrate brain. Synapses communication and brain evolution in physiological and pathological conditions is a theme that is currently inspiring our Department.

As newly appointed director, it is for me the first occasion to welcome you at the DNF, a department with a strong history in Neuroscience. This is a moment of change – new research groups have recently joined the Department and additional ones will join in the coming years. I am therefore delighted by your presence and happy to share with you such an important moment for the DNF supported with enthusiasm by the entire staff of the DNF.

Enjoy the Symposium and let the music play and the synapses shape!

Claudia Bagni

3rd DNF Symposium: Evolution of the Brain

Program

- 8h45 - 9h00: Opening Remarks (Egbert Welker)
- 9h00 - 9h40: **Wieland Huttner**, Max Planck Institute of Molecular Cell Biology and Genetics, Dresden, Germany
Neural Stem and Progenitor Cells and Neocortex Expansion in Development and Evolution
- 9h40 - 10h20: **Leah Krubitzer**, Center of Neuroscience, University of California, Davis, USA
How does evolution build a complex brain?
- 10h20 - 11h00: Morning Break
- 11h00 - 11h40: **Robin Dunbar**, Department of Experimental Psychology, University of Oxford, UK
What's so social about the social brain?
(Introduced by Laurent Keller)
- 11h40 – 11h55: **Jonathan Moss**, DNF
NMDA receptors of radial glia-like adult neural stem cells regulate their proliferation, differentiation and morphology (Poster 19)
- 11h55 – 12h10: **Denise Becker**, DNF
Neuroinflammatory TNF α Impairs Memory via Astrocyte Signaling (Poster 18)
- 12h10 – 12h25: **Nuria Domínguez-Iturza**, DNF
CYFIP1 orchestrates axonal development and brain wiring: insights into ASDs (Poster 9)

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12h25 - 14h00: Poster Session & Lunch

14h00: Music: Opus 4 – string quartet

14h30 - 15h10: **Dirk Fasshauer**, Department of Fundamental Neurosciences, University of Lausanne, Switzerland

Evolution of the neuronal secretion apparatus

15h10 - 15h50: **Richard Benton**, Center for Integrative Genomics, University of Lausanne, Switzerland

Evolution of olfactory circuits

15h50 - 16h30 Afternoon Break

16h30 - 17h10: **Gáspár Jékely**, Max Planck Institute for Developmental Biology, Tübingen, Germany

Simple sensory-motor circuits in a zooplankton larva and evolution of neural circuits

17h10 - 17h30: Poster Prizes & Closing Remarks (Claudia Bagni)

Followed by: The DNF Brain Party – drinks, food & music

Musical contributions of

Quatuor op.4 (conservatoire de Lausanne; class of Hans Egidi)

Samuel Hirsch, violon

Léa Al Saghir, violon

Son Pham Ba, alto

Marie Ausländer, violoncelle

Duo Dlam (Marimba - Percussion)

Annick Richard

Luca Musy

YMTA (groupe *a cappella* des étudiant·e·s en médecine):

Marie Schmidhauser

Yohan Guichoud

Antsa Randretsanilo

Tiary Randretsanilo

DJ

Alexandre Croquelois

Speakers



Richard Benton

Center for Integrative Genomics,
University of Lausanne, Switzerland

Born in Edinburgh, Richard Benton's studies took him to Cambridge where he obtained his PhD in biology. He worked as a post-doctoral fellow at The Rockefeller University in New York between 2003 and 2007. He then joined the Center for Integrative Genomics at the University of Lausanne as an assistant professor and became associate professor in 2012. Richard Benton studies sensory perception in the model organism *Drosophila melanogaster*, the common vinegar fly. He wants to decipher the molecular logic of how insects receive chemical signals to distinguish kin, mates, competitors, prey and predators. This involves identifying the receptors in the nose and the neurons in the brain that respond to information insects receive via their sense of smell. Benton tries to understand how a specific substance triggers activity in certain regions of the brain to provoke particular behaviors.

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Robin Dunbar

Department of Experimental Psychology, University of Oxford, UK

Robin Dunbar gained his PhD from Bristol University. He is currently Professor of Evolutionary Psychology at the University of Oxford. He has held Research Fellowships and Professorial Chairs in Psychology, Biology and Anthropology at the University of Cambridge, Stockholm University, University College London, and the University of Liverpool. He is an elected Fellow of the British Academy, and co-Director of the British Academy's Centenary Research Project. His principal research interests focus on the evolution of sociality in mammals (with particular reference to ungulates, primates and humans). He is best known for the social brain hypothesis, the gossip theory of language evolution and Dunbar's Number (the limit on the number of relationships that we can manage). His current project focuses on the mechanisms of social cohesion, and uses a range of approaches from comparative analysis to cognitive experiments to neuroimaging to explore the mechanisms that allow humans to create large-scale communities.

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Dirk Fasshauer

Department of Fundamental Neurosciences,
University of Lausanne, Switzerland

Dirk Fasshauer studied biology and received his doctorate at the University of Göttingen in 1994. He worked as a post-doctoral fellow at Yale University from 1995 to 1997 and moved from there to the Max Planck Institute for Biophysical Chemistry. From 2002, he headed the “Structural Biochemistry” Research Group in the Department of Neurobiology. In 2009, he joined the Department of Cell Biology and Morphology, now the Department of Fundamental Neurosciences, at the University of Lausanne as an associate professor. His group focuses on a structural and functional description of the protein machinery that drives the calcium-dependent release of neurotransmitters from synaptic vesicles. Ultimately, he is interested in the evolutionary development of the machinery because its core elements are highly conserved among all eukaryotes and throughout the cell.

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Wieland Huttner

Max Planck Institute of Molecular Cell Biology and Genetics, Dresden, Germany

Wieland Huttner studied medicine in Oxford and Hamburg, where he obtained his doctorate in 1976. After a post-doctorate at the Max-Planck-Institute for Experimental Medicine in Göttingen and at Yale University, he returned to Germany in 1981, where he headed a junior research group at the Max-Planck-Institute of Psychiatry in Munich. In 1985, he became a group leader at the EMBL in Heidelberg and in 1991 he was appointed as Professor and Chair of the Institute for Neurobiology at the University of Heidelberg. Currently he is Director and Research Group Leader at the Max-Planck-Institute of Molecular Cell Biology and Genetics in Dresden, Germany. His research focuses on the molecular and cellular mechanisms underlying the evolutionary expansion of the neocortex. His research group compares the molecular characteristics of neural stem and progenitor cells in liss- and gyrencephalic mammalian species to obtain insight into their differential proliferative potential. This molecular approach has led to the discovery of various genes involved in the neocortical expansion during mammalian evolution.

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Gáspár Jékely

Max Planck Institute for Developmental Biology,
Tübingen, Germany

Gáspár Jékely obtained his PhD in genetics at the Eötvös Loránd University in Budapest. He then worked as a post-doctoral fellow at the EMBL in Heidelberg from 2000 to 2002 in the laboratory of Pernille Rorth and moved from there as Junior fellow to the Institute for Advanced Study at the Collegium Budapest. In 2004, he moved again to the EMBL in Heidelberg to work in the laboratory of Detlev Arendt. Since 2007, Gáspár Jékely is leading a Research Group at the Max-Planck-Institute for Developmental Biology in Tübingen. Using an interdisciplinary approach, his research aims at identifying the molecular and circuit bases of behavior in a marine invertebrate model organism, the annelid *Platynereis*. As a 'living fossil', *Platynereis* can help to shed light on how animal nervous systems evolved at the dawn of animal life and diversified during the 'Cambrian explosion', one of the most spectacular events in the history of life.

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Leah Krubitzer

Evolutionary Neurobiology, University of California, Davis, USA

Leah Krubitzer is Professor at the Department of Psychology and Center for Neuroscience at the University of California, Davis. She attended Pennsylvania State University where she received a BS degree, and did her graduate work at Vanderbilt University, where she received her PhD in Physiological Psychology. She spent over six years in Australia, at the University of Queensland where she studied a number of primitive mammals including the duckbilled platypus and the spiny ant eater. Leah Krubitzer is interested in how complex brains, such as those in humans, are built from simpler forms. Her work examines the anatomical connections and electrophysiological properties of neurons in the neocortex, the portion of the brain responsible for perception, cognition, learning, and memory. Through comparative studies, it is possible to determine which features of the neocortex are shared by all mammals, and how new features have been added during morphological or behavioral specialization. In this way, she can reconstruct the evolution of the neocortex and its relationship to functional changes. Her work accounts for the remarkable diversity in mammalian behavioral and perceptual abilities through the action of a few evolutionarily old developmental mechanisms. While constraining evolutionary change, these mechanisms have also provided the variation needed for such diversification.

Poster abstracts

I. Molecular recognition and evolution of olfactory receptors

Benoîte Bargeton, Matteo Dal Peraro and Richard Benton

Centre for Integrative Genomics, University of Lausanne

Chemosensation allows animals to detect and respond to myriad chemical signals in their environment indicating food, dangers, kin and mates. A new chemosensory receptor repertoire, the Ionotropic Receptors (IRs), which have derived from ionotropic glutamate receptors (iGluRs), was recently characterized. IRs are expressed in chemosensory organs across protostomes, including insects, *C. elegans* and *Aplysia*. Functional characterization in *Drosophila* has shown that they mediate recognition of structurally-diverse acids and amines. Like iGluRs, IRs function as ligand-gated ion channels, composed of heteromeric complexes of a canonical IR, important for defining ligand specificity, and a co-receptor (either IR8a or IR25a). Study of the “Venus fly-trap” ligand binding domain (LBD) of IRs provides an important model to understand the molecular basis of chemosensory recognition, and how this has evolved from the glutamate-recognition properties of iGluRs. I have investigated IRs LBD structure in silico by comprehensive protein modeling. This analysis combined with evolutionary-guided structure function studies of IR75a and IR75b, activated by one to six carbon long carboxylate, and the co-expression of IR8a and IR75a or IR75b in *Xenopus laevis* oocytes provided evidences for convergent mechanisms for changes in ligand-specificity in this family of olfactory receptors. The variant amino acids in the ligand binding pocket (four divergent positions between IR75b from *D. melanogaster* and *D. sechellia* and three between IR75as) confer partial to full properties to their agonists. Combined approaches in silico, in vitro and with collaborators in vivo provide insight into the evolution of an ancient ligand-binding module of glutamate receptors.

2. Evolution of acid-sensing olfactory circuits in drosophilids

Lucia L. Prieto-Godino, Raphael Rytz, Steeve Cruchet, Benoîte Bargeton, Liliane Abuin, Ana F. Silbering, Vanessa Ruta, Matteo Dal Peraro and Richard Benton

Centre for Integrative Genomics, University of Lausanne

Animals adapt their behaviors to specific ecological niches, but the underlying genetic and cellular basis of nervous system evolution is poorly understood. We have compared the olfactory circuits of the specialist *Drosophila sechellia* – which feeds exclusively on *Morinda citrifolia* fruit – with its generalist cousins *D. melanogaster* and *D. simulans*. We find *D. sechellia* exhibits novel odor-evoked attraction and physiological sensitivity to the abundant *Morinda* volatile, hexanoic acid, and characterize how the responsible sensory receptor (the variant ionotropic glutamate receptor IR75b) and attraction-mediating circuit have evolved. A single amino acid change in IR75b is sufficient to recode it as a hexanoic acid detector. Expanded representation of this sensory pathway in the brain relies on additional changes in the IR75b promoter and trans-acting loci. By contrast, higher-order circuit adaptations are not apparent, suggesting conserved central processing. Our work links olfactory ecology to structural and regulatory genetic changes influencing nervous system anatomy and function.

**3. Emergence of cell-cell communication in animals:
Feeding mechanism in *Trichoplax adhaerens***

Jules Duruz, Jean Daraspe, Thomas Krueger, Stephane Escrig, Dirk Fasshauer, Anders Meibom, Bruno Humbel, Frederique Varoquaux

Département des Neurosciences Fondamentales, Université de Lausanne, Rue du Bugnon 9, 1005 Lausanne

With its remarkably simple morphological organization - lacking polarity and organs, circulatory, digestive or nervous system – *Trichoplax adhaerens* (T.ad) can be viewed as a "minimalist metazoan". Sole identified member of the phylum Placozoa (Schulze, 1883), it is presented by most researchers together with sponges as the earliest diverging animal, yet displays a complex array of behaviors (Heyland et al., 2014) and expresses proteins associated to major biological processes, including neural patterning, neuro-secretion and synapse formation (Srivastava et al., 2008). Unraveling the physiology of T.ad and its phylogenetic relationship with other basal metazoans is essential to understand the emergence of cell-cell communication and reconstitute the evolution of the basic animal body plan. The current project proposes to uncover its mode of nutrition (uptake and exchange between different cells) using classical imaging, Transmission Electron Microscopy and Nanoscale Secondary Ion Mass Spectrometry (NanoSIMS).

4. Analysis of Scfd2 - a new member of the SM protein family

Janeta Jordanova and Dirk Fasshauer

Département des Neurosciences Fondamentales, Université de Lausanne, Rue du Bugnon 9, 1005 Lausanne

Proteins of the SM (Sec1/Munc18) protein family are indispensable regulators of vesicle fusion in eukaryotic cells. Currently, four basic types of SM proteins are known: Sec1 or Munc18, Sly1, Vps45 and Vps33. In different trafficking steps, they interact with respective SNARE (soluble N-ethylmaleimide-sensitive factor attachment receptor) proteins, which catalyze the fusion of vesicle and target membranes by zippering into a tight four-helix bundle between membranes. Most SM proteins interact directly with the Qa-SNARE or syntaxin, although different binding modes have been proposed. Upon phylogenetic analysis of the SM protein family, we came across a new member of the family, named Scfd2 (Sec1 Family Domain Containing 2). Although it is likely that this new SM protein type was present in the genome of the last eukaryotic common ancestor, it has only been maintained in certain lineages, among them most animals and plants. It is absent, however, from several widely used model organisms like *S. cerevisiae*, *C. elegans* and *Drosophila*. Little is known about the function of Scfd2 and it is not clear whether it acts in vesicle trafficking in a manner comparable to other SM proteins and whether it has a specific SNARE binding partner. Recent data suggest that Scfd2 may interact or even be a part of a tethering complex involved in ER-Golgi transport. In order to shed light on the function of Scfd2, we are investigating its interaction with the key proteins involved in ER-Golgi trafficking, using biochemical approaches.

5. Evolutionary conserved pattern of SNARE proteins could explain binding preferences of cognate complexes

Mykola Dergai, Nickias Kienle, Emilie Neveu, Tobias Klöpffer and Dirk Fasshauer

Département des Neurosciences Fondamentales, Université de Lausanne, Rue du Bugnon 9, 1005 Lausanne

Transport of cargo between organelles in eukaryotic cells is mediated by vesicles that bud from a donor compartment and specifically fuse with an acceptor membrane. Currently, it is becoming clear that the underlying molecular machineries involved in the principal aspects of vesicular trafficking are highly conserved, not only between different species but also between different vesicle trafficking steps. In all steps, the central machinery involved in the fusion process is composed of members of the SNARE protein family. Their defining feature is an extended coiled-coil stretch. Distinctive, heterologous sets of SNARE proteins anchored in the vesicle and target membrane assemble in a zipper-like fashion into a four-helix bundle, providing the energy to fuse membranes. During SNARE zippering, 16 layers of mostly hydrophobic residues are formed. These layer residues are highly conserved. Our earlier computational investigations have shown that SNARE proteins split into four basic types, reflecting their position in the four-helix bundle complex. Among these four basic types, we had established 20 SNARE subclasses that probably represent the original repertoire of a eukaryotic ancestor. The subtypes can assemble only into few distinct SNARE complexes that work in trafficking between the major compartments of the endomembrane system. It has remained unclear, however, how specificity of SNARE complex formation is achieved. We now show that residues forming the hydrophobic core are sufficient to identify SNARE proteins among all other proteins. These residues are also sufficient to discriminate SNARE subfamilies relevant to distinct trafficking steps. Moreover, we noticed that the hydrophobic layer residues form conserved geometrical patterns that might permit only cognate SNARE assemblies to form.

6. Reduced CYFIP disrupts mitochondrial metabolism in GABAergic neurons provoking neuropsychiatric-like behavior

Alexandros K. Kanellopoulos, Vittoria Mariano, Marco Spinazzi, Ulrike Pech, Ka Wan Li, August B. Smit, Andre Fiala, Patrick Callaerts and Claudia Bagni

KU Leuven, Center for Human Genetics and Leuven Research Institute for Neuroscience and Disease (LIND), Leuven, Belgium. VIB Center for the Biology of Disease, Leuven, Belgium. Département des Neurosciences Fondamentales, Université de Lausanne, Rue du Bugnon 9, 1005 Lausanne

Deletions, duplications and copy number variations (CNVs) of the CYFIP1 gene predispose to neurodevelopmental and neuropsychiatric disorders. Here we show that reduced *Drosophila* CYFIP levels lead to deficits in social interaction such as competition for food and courtship, two ASDs-like phenotypes in flies. Reduction of dCYFIP causes an excess of protein synthesis. An excess of GABA-A receptor level and reduced neuronal Ca²⁺ activity were observed in the fly brain. Importantly, we show that altered mitochondrial metabolism in GABAergic neurons causes disrupted social behavior. The increased mitochondrial activity was brain-specific in flies. Genetic and pharmacological manipulations of dCYFIP deficiency or mitochondrial metabolism restore molecular, cellular and social deficits. Our findings pave the way for the understanding of the molecular mechanism/s underlying abnormal social behavior and support the current hypothesis of mitochondrial dysfunctions in neuropsychiatric disease as autism spectrum disorders and schizophrenia.

7. Drosophila models of human neurodevelopmental diseases

Mariano Vittoria, Vannelli Anna and Claudia Bagni

Laboratory for Molecular Neurobiology, VIB Center for the Biologie of Disease, K.U. Leuven - Center for Human Genetics Herestraat 49 bus 602, 3000 Leuven, Belgium

The most frequent form of inherited intellectual disability and autism is the Fragile X Syndrome, caused by the absence of the RNA binding protein FMRP. Individuals with FXS display a range of developmental and behavioural deficits with mild to severe ID, attention deficit disorder and autism. FMRP interacts with the cytoplasmic FMRP-interacting protein (CYFIP1). In brain, CYFIP1 is involved in two molecular processes crucial for synaptic development and absence of this protein leads to dendritic spine dysgenesis. Therefore, binding FMRP, it regulates the translation of FMRP-targeted mRNAs and as part of the WAVE regulatory complex it regulates actin remodelling. Several studies report that copy number variation of CYFIP1 gene are linked to several neuropsychiatric disorders such as autism spectrum disorder, schizophrenia, epilepsy and Alzheimer's disease. In our laboratory, following different approaches such as behavioral assays, electrophysiology, molecular tools and immunohistochemistry, we study the function of FMRP and CYFIP1 using *Drosophila melanogaster* as model organism. *dfmr1* and *dcyfip* homologs have been reported to be involved in synapse formation and stability, in neuromuscular junction development and axonal branching. Here we show that *dfmr1* and *dcyfip* mutants show behavioral impairments such as circadian rhythm, social interaction and locomotion deficits. In addition, we observed impairments in synaptic transmission at the neuromuscular junction in larvae and both phototransduction and transmission between the photoreceptor and lamina neurons in retina are altered. These findings make *Drosophila* a useful model for studying the molecular, and cellular functions of FMRP and CYFIP and to investigate their role in different neuropsychiatric disorders.

8. Genome-wide identification of functional neuronal activity-dependent FXR2P targets

Nicholas Rajan, Adrian C. Lo, Esperanza Fernandez, Mark Fiers, Tilmann Achsel and Claudia Bagni

Département des Neurosciences Fondamentales, Université de Lausanne, Rue du Bugnon 9, 1005 Lausanne

The Fragile X Related Protein 2 (FXR2P) is a member of a small family of RNA-binding proteins that includes the Fragile X mental retardation protein (FMRP). FXR2P shares structural and functional similarities with FMRP, such as RNA binding, ribosome association and nucleocytoplasmic shuttling. Although it is known that loss of FXR2P causes neuronal and behavioral deficits in mice, the function of FXR2P and its mRNA target repertoire are largely unknown. Here we report the identification, characterization and validation of functionally relevant FXR2P target mRNAs in brain. Using RNA-immunoprecipitation followed by high-throughput sequencing (RIP-Seq), we identified 390 FXR2P-bound mRNAs. Network enrichment analysis showed that FXR2P preferentially binds mRNAs involved in regulation of neuronal contact and activity. The majority of FXR2P-copurifying mRNAs show a marked overlap with the mRNA targets of Fragile X Mental Retardation Protein in the brain, suggesting a functional convergence between the fragile X proteins. We show that absence of FXR2P impairs activity-dependent de novo protein synthesis mediated through the BDNF-TrkB signaling pathway. Of note, a significant number of the FXR2P mRNA repertoire is associated with autism, epilepsy and schizophrenia. Our findings shed light on the brain regulatory network orchestrated by FXR2P and highlight a role of FXR2P in brain development and neuro-developmental dysfunctions.

9. CYFIPI orchestrates axonal development and brain wiring: insights into ASDs

Nuria Domínguez-Iturza, Ana Rita Santos, Esperanza Fernandez, Disha Shah, Zsuzsanna Callaerts-Vegh, Tilmann Achsel, Annemie Van Der Linden, Rudy d'Hooge and Claudia Bagni

Département des Neurosciences Fondamentales, Université de Lausanne, Rue du Bugnon 9, 1005 Lausanne

Deletions and duplication of the 15q11.2 chromosomal region have been recently associated with autism spectrum disorder (ASD), schizophrenia and other intellectual disabilities. Among the genes located in this region, Cytoplasmic FMRP-Interacting Protein 1 (CYFIPI) is known to be the strongest candidate for several neurodevelopmental disorders. Using a mouse model for the CYFIPI deletion (*Cyfp1^{+/-}*), we unravelled the role of CYFIPI in brain development and functional connectivity. We found that CYFIPI levels are required for a correct neuronal migration and cortical layering formation during early stages of development. Additionally, reduced levels of CYFIPI compromise axonal growth and pathfinding in vivo, leading to abnormal white matter architecture and an overall reduction in functional connectivity in young animals. Importantly, *Cyfp1^{+/-}* mice show impaired social behavior, resembling some of the clinical features observed in patients with ASD. Our findings highlight the critical role of CYFIPI during embryonic brain development and network formation, correlating these early developmental defects with a reduced functional connectivity and social behavior, and ultimately providing cellular, molecular and behavioral evidence in support of CYFIPI as a strong candidate gene for ASDs.

10. FMRP needs different partners for the regulation of subsets of mRNAs

Laura D'Andrea, Giorgia Pedini, Esperanza Fernández, Tibor Pastor, Maria Giulia Farace, Tilmann Achsel and Claudia Bagni

University of Rome Tor Vergata, Department of Biomedicine and Prevention, 00133 Rome

The Fragile X Mental Retardation Protein (FMRP) is the protein responsible for the Fragile X Syndrome (FXS), the most frequent form of inherited intellectual disability. FMRP is an RNA binding protein that regulates local translation, mRNA stability, or transport of specific mRNAs. FMRP binds more than 4% of transcripts in mouse brain, but its action on the majority of them is still unknown. Here we show that FMRP together with FXR2 binds PSD95 mRNA and the dimer increases its translation (1). To bind to another subset of mRNAs, FMRP requires SMN. Binding of this dimer decreases expression of the respective proteins. Finally, miR-29a could mediate the role of FMRP in the regulation of CTNNB1 mRNA during neuronal development. Our data suggest that there are different FMRP complexes, and each determines the fate of a subset of mRNAs in a different way, defining the type of regulation played by FMRP in a precise spatial-temporal window.

(1) Fernández et al., 2015. *J. Neuroscience* 35, 9402-9408

II. Spinal Cord T-Cell Infiltration in the Rat Spared Nerve Injury Model: A Time Course Study

Christophe Gattlen, Christine B. Clarke, Nicolas Piller, Guylène Kirschmann, Marie Pertin, Isabelle Decosterd, Romain-Daniel Gosselin and Marc R. Suter

Département des Neurosciences Fondamentales, Université de Lausanne, Rue du Bugnon 9, 1005 Lausanne

The immune system is involved in the development of neuropathic pain. In particular, the infiltration of T-lymphocytes into the spinal cord following peripheral nerve injury has been described as a contributor to sensory hypersensitivity. We used the spared nerve injury (SNI) model of neuropathic pain in Sprague Dawley adult male rats to assess proliferation, and/or protein/gene expression levels for microglia (Iba1), T-lymphocytes (CD2) and cytotoxic T-lymphocytes (CD8). In the dorsal horn ipsilateral to SNI, Iba1 and BrdU stainings revealed microglial reactivity and proliferation, respectively, with different durations. Iba1 expression peaked at D4 and D7 at the mRNA and protein level, respectively, and was long-lasting. Proliferation occurred almost exclusively in Iba1 positive cells and peaked at D2. Gene expression observation by RT-qPCR array suggested that T-lymphocytes attracting chemokines were upregulated after SNI in rat spinal cord but only a few CD2/CD8 positive cells were found. A pronounced infiltration of CD2/CD8 positive T-cells was seen in the spinal cord injury (SCI) model used as a positive control for lymphocyte infiltration. Under these experimental conditions, we show early and long-lasting microglia reactivity in the spinal cord after SNI, but no lymphocyte infiltration was found.

12. Extracellular potassium modulates glial glutamate uptake: effects on excitatory neurotransmission

*Anne-Bérengère Rocher**, *Theresa S. Rimmele**, *Joel Wellbourne-Wood* and *Jean-Yves Chatton*

Département des Neurosciences Fondamentales, Université de Lausanne, Rue du Bugnon 9, 1005 Lausanne. *equal contributions.

During neuronal activity, extracellular K^+ ($[K^+]_{out}$) significantly increases during the repolarization of firing neurons. Glutamate is also released in the synaptic cleft during action potentials and cleared out mainly by sodium-dependent glutamate transporters, such as GLT-1, at the astrocyte membrane. The glutamate transporter stoichiometry includes the uptake of one glutamate together with the extrusion of one K^+ in the extracellular space. In this study, we show with microspectrofluorimetry in primary astrocytes that altering $[K^+]_{out}$ had a strong, immediate, and reversible effect on glutamate transport. Low $[K^+]_{out}$ enhanced transport activity, whereas high $[K^+]_{out}$ markedly diminished glutamate capture. The latter effect was also observed by measuring glutamate transporter currents in voltage-clamped HEK293 cells expressing GLT-1, and astrocytes in acute mouse brain slices. Several independent studies previously demonstrated that pharmacological inhibition of glutamate uptake operated a negative feedback on neuronal transmission by causing extrasynaptic glutamate spillover and subsequent activation of presynaptic neuronal group II mGluRs. We thus tested whether $[K^+]_{out}$ and its modulatory effects on astrocyte glutamate transport could be the physiological trigger for this feedback mechanism. We found that increasing $[K^+]_{out}$ from 3 to 6mM caused a reversible decrease in neuronal mEPSC frequency that was prevented by group II mGluR inhibition using LY-341495. These findings reveal a novel negative feedback cascade linking $[K^+]_{out}$ elevation, glutamate transporter inhibition, and presynaptic mGluR activation. This mechanism could be fundamental for keeping excitatory neuronal activity under control.

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13. Development and Characterization of an Extracellular Potassium Nanosensor

Joel Wellbourne-Wood and Jean-Yves Chatton

Département des Neurosciences Fondamentales, Université de Lausanne, Rue du Bugnon 9, 1005 Lausanne

Changes in the extracellular concentration of potassium ($[K^+]_o$) have a large functional impact on the brain; for example they affect membrane potential, K^+ equilibrium potential, and synaptic transmission. Increases in $[K^+]_o$, such as those which occur during neuronal activity, are largely controlled for by astrocytes, since these cells are in charge of maintaining K^+ homeostasis through the uptake of K^+ from the extracellular space and its redistribution through the syncytium. In addition, K^+ has been shown to play a role in several more complex processes, such as modulating glutamate transporter activity and stimulating astrocytic glycolysis. To better understand these $[K^+]_o$ fluctuations and to gather spatiotemporal data regarding K^+ related events we developed an extracellular potassium sensitive fluorescent nanosensor. Our nanosensor design consists of an ion sensitive fluorescent dye encapsulated in a nanoparticle. Here we report on the generation and characterization of the nanosensor, including its spectral properties, its sensitivity, and its selectivity for K^+ and Na^+ . The in-vitro characterizations of the nanosensor's kinetics, as well as its stability and sensitivity for two photon imaging of $[K^+]_o$ in brain tissue demonstrates the effectiveness of the nanosensor strategy. This novel approach of optical imaging of $[K^+]_o$ paves the way for future investigations into a wide range of $[K^+]_o$ mediated effects.

14. Evaluation of the receptor-mediated function of lactate in neuronal activity

Haïssa de Castro Abrantes, Anne-Béregère Rocher, Céline Schmuziger, Stefan Offermanns, Julien Puyal, Jean-Yves Chatton

Département des Neurosciences Fondamentales, Université de Lausanne, Rue du Bugnon 9, 1005 Lausanne

The recent discovery of a Gi protein-coupled receptor for lactate in neurons of the central nervous system, called hydroxycarboxylic acid receptor 1 (HCAIR), has pointed to additional non-metabolic effects of lactate on neuronal network activity. However, the expression pattern of HCAIR in the brain is still unclear. In addition, the absence of an antagonist for HCAIR makes it difficult to evaluate its functional role. In this study, we present evidence that HCAIR is present and functional in the brain. By assessing the HCAIR mRNA transcript expression in mice using qRT-PCR, we showed that HCAIR is indeed present in several brain regions. In a reporter mouse line which expresses a red fluorescent protein (mRFP) under the control of the HCAIR promoter, we could identify different brain regions in which HCAIR is expressed. At the functional level, L-lactate and 3,5-DHBA, a non-metabolized agonist of HCAIR, reversibly decreased by 40% spontaneous spiking activity of primary cortical neurons of wild-type mice. Neither compounds affected the activity of neurons prepared from HCAIR knock-out animals, which demonstrates the requirement of HCAIR activation and the non-metabolic nature of the lactate effects on neuronal activity. These results provide strong support for the expression of HCAIR in the mouse brain and for its role as modulator of neuronal network activity.

15. Mutations in Eml1 induce functional and structural changes in HeCo mutant mouse neurons: an animal model for cortical malformation displaying epilepsy

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In the Heterotopic Cortex (HeCo) mouse model, the expression of the mutated echinoderm microtubule associated protein-like 1 (EML1) leads to ectopic cortical progenitors during embryogenesis developing into neuronal heterotopia and, ultimately epilepsy in the adult mouse. This phenotype is reminiscent of the pathology observed in human suffering from malformation of cortical development (MCD). Such MCDs are observed in 14% of all epileptic cases and in 40% of severe or intractable epileptic cases in children. The aim of this study is to examine the effects of mutated EML1 on the electrophysiological and morphological properties of cortical neurons. We used whole-cell patch-clamp recordings and biocytin filling of pyramidal cells in cortical slices prepared from HeCo and wild-type (WT) littermate mice. The resting membrane potential (V_r) of neurons from HeCo mice was unchanged when compared to that of WT. However, evoked repetitive action potential (AP) firing rates were increased by 3.3 fold in mutant neurons. In addition, HeCo cells display HCN-mediated currents blocked by ZD-7288 and absent from WT neurons. The rheobase was lower in HeCo neurons vs WT (41%). These data suggest that HeCo neurons are more excitable. The preliminary morphological analysis of electrophysiologically characterized HeCo cells exhibited disoriented cell bodies and a global multipolar aspect. In conclusion, we showed that expression of mutated Eml1 results in functional and structural changes in HeCo neurons which might be at the origin of intractable epilepsy linked to cortical malformation.

16. Glutamatergic synaptic recruitment of thalamic reticular nucleus by postsubicular afferents: role in head-direction system?

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The thalamic reticular nucleus (TRN) is a cornerstone of attentional mechanisms as it modulates state-dependent interactions between thalamic circuits and sensory cortices. The anterior portion of the TRN is connected to limbic structures, yet little is known about its role in thalamocortical communication. Identification of novel afferents to the TRN could bring insight on unknown functions of this strategically positioned nucleus. Retrobead and Phaseolus injections in mouse brains revealed postsubiculum (PSub) projections to the anterior portion of both TRN and thalamus (AThal). TRN and AThal neurons discharge in a time-locked manner in response to postsubicular afferent activation in *in vitro* acute brain slices. Minimal optogenetic stimulation revealed a four-fold greater connectivity at PSub-TRN synapses than at PSub-AThal. Both afferents showed little short-term plasticity, even during train stimulation, which lead to persistent membrane depolarization and enhanced/maintained discharge propensity. In urethane-anesthetized mice, light stimulation of the PSub triggered rebound burst firing in TRN neurons. Freely moving *in vivo* data showed different light-evoked responses along the postsubicular-thalamic axis: fast excitation, delayed excitation, suppression of firing. Together, these data identify a faithful information transfer between PSub and thalamic circuits, including TRN. The AThal/PSub circuit is involved in the Head Direction (HD) signal, an essential component of the self-referenced navigation system. Neurons encoding for HD are most prominently found within these two structures. Although the TRN controls all dorsal thalamus through feedforward inhibition, its role in the HD system remains largely unexplored and will be investigated in this study.

17. VMAT2 in astrocytes regulates morphology of pyramidal neurons in developing PFC by modulating extracellular levels of dopamine

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Neuromodulation of neuronal circuits of the prefrontal cortex (PFC) by monoamines influences synaptic plasticity and executive functions and may play critical roles in psychiatric disorders (Arnsten et al., *Neuron*, 2012). The cellular mechanisms governing the homeostasis of monoamines in the developing PFC are not completely defined. Evidence that astrocytes express the whole enzymatic apparatus for the metabolism of monoamines (Youdim et al., *Nature Rev Neurosci*, 2006) strongly suggests a possible involvement of astroglial cells in mechanisms governing monoaminergic homeostasis. Here we report that astrocytes located in PFC express a plasma membrane organic cation transporters 3 (OCT-3) and contain dopamine (DA). Moreover we found that astrocytes express vesicular monoamine transporter 2 (VMAT2) and that the signal of this transporter was readily recognizable on different subset of intracellular organelles such as endosomes and lysosomes. The physiological relevance of VMAT2 in astrocytes is investigated by generating conditional transgenic mice in which VMAT2 is specifically deleted in GFAP expressing cells (aVMAT2cKO). Interestingly, VMAT2 deletion in astrocytes leads to a specific deficit in astrocytes storing DA and a decrease in the extracellular levels of DA in the PFC. Moreover, we found out that the decreased levels of DA in the developing PFC of aVMAT2cKO mice alters the spine formation of pyramidal neurons that can be rescued by chronic administration of L-DOPA or VMAT2 re-expression in astrocytes by lentivirus during postnatal development. These results highlight a critical role for VMAT2 in astrocytes in the regulation of DA levels and the normal development of pyramidal neurons in the PFC.

18. Neuroinflammatory TNF α Impairs Memory via Astrocyte Signaling

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TNF α , a proinflammatory cytokine, is constitutively present in the CNS at low levels and upregulated during neuroinflammation. It participates in synaptic plasticity, regulating AMPAR trafficking (Stellwagen and Malenka, *Nature*, 2006) and astrocyte-dependent activation of pre-synaptic NMDAR (Santello et al., *Neuron*, 2011). Local increase of TNF α during CNS inflammation could alter its control on the hippocampal synaptic circuits and lead to the insurgence of cognitive disturbances. To directly test if neuroinflammatory TNF α causes the insurgence of a cognitive phenotype we used a mouse model of multiple sclerosis (MS), an inflammatory CNS pathology in which patients suffer from cognitive impairment related to hippocampal dysfunction (Benedict and Zivadinov, *Nat Rev Neurol*, 2011). We provide direct evidence that neuroinflammatory TNF α causes cognitive impairment via astrocyte signaling (Habbas et al., *Cell*, 2015). Thus, in the MS mice we observed: (a) reduction of contextual memory; (b) persistent alteration of the EC-GC synapse in the hippocampus, a critical component of the context-dependent fear response (Liu et al., *Nature*, 2012); with (c) local inflammation with increased TNF α levels. Using a novel transgenic model we linked the above observations and demonstrated that synaptic and memory alterations depend on TNF α acting via astrocyte TNFR1 signaling, as they were largely reconstituted upon conditional re-expression of TNFR1 solely in astrocytes. The identified mechanism may contribute to the insurgence of a cognitive phenotype in MS, and in other CNS conditions associated with inflammation.

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19. NMDA receptors of radial glia-like adult neural stem cells regulate their proliferation, differentiation and morphology

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Neurogenesis in the adult brain supplies the dentate gyrus with new neurons that integrate into the hippocampal circuit throughout adult life, affecting functions such as learning and memory. The adult neural stem cells, which give rise to these new neurons, possess a unique morphology that positions their fine processes in direct apposition to excitatory synapses of the molecular layer. No synapses appear to be formed with these fine processes, but recent studies have shown that these stem cells can be responsive to N-methyl-D-aspartate (NMDA), raising the possibility that they possess extra-synaptic glutamate receptors. Using ultrastructural analyses, at the electron microscope level, we show that radial glia-like (RGL) neural stem cells of the hippocampal neurogenic niche possess immunogold labelling for NR1; the obligatory subunit of the NMDA receptor. This extra-synaptic labelling is sparser than that of neighbouring granule neurons, but at significantly higher densities than negative controls. It suggests that RGL neural stem cells can receive a glutamatergic signal, via spill-over from apposing synapses, which could influence their activation to divide and differentiate in to new neurons. In order to investigate this possibility further, we knocked down the NR1 subunit specifically in RGL neural stem cells using Cre::Ert2 technology. Our data suggests that the NMDA receptor not only regulates the proliferation of the RGL neural stem cell, but also affects its morphology and fate. Taken together, these results show the presence of NMDA-receptors on the RGL neural stem cell, and their involvement in its proliferation, differentiation and morphology.

20. SHANK3 controls maturation of social reward circuits in the VTA

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Haploinsufficiency of SHANK3, encoding the synapse scaffolding protein SHANK3, leads to a highly penetrant form of Autism Spectrum Disorder (ASD). How SHANK3 insufficiency affects specific neural circuits and this is related to specific ASD symptoms remains elusive. Here we used shRNA to model Shank3 insufficiency in the Ventral Tegmental Area (VTA) of mice. We identified dopamine (DA) and GABA cell-type specific changes in excitatory synapse transmission that converge to reduce DA neuron activity and generate behavioral deficits, including impaired social preference. Administration of a positive allosteric modulator of the type I metabotropic glutamate receptors (mGluRI) during the first postnatal week restored DA neuron excitatory synapse transmission and rescued the social preference defects, while optogenetic DA neuron stimulation was sufficient to enhance social preference. Collectively, these data reveal the contribution of impaired VTA function to social behaviors and identify mGluRI modulation during postnatal development as a potential treatment strategy.

21. Lactate metabolism and neuroprotection in mouse MCAO using hyperpolarized ¹³C-lactate: a preliminary study

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Stroke is a major public health challenge in the context of the current demographic changes. Lactate is a known neuroprotectant in stroke at the preclinical level. Among a wide range of applications, hyperpolarized (HP) magnetic resonance enables in vivo real-time measurement of biochemical transformations of HP ¹³C-labeled precursors including lactate. 30 min middle cerebral artery occlusion (MCAO) was induced in male C57BL/6 mice with the suture model under laser Doppler blood flow monitoring. 2 hr post reperfusion, mice were placed into a 9.4 T animal scanner. Morphological changes were detected by T2W MRI images indicating the appearance of ischemia in striatum only in one hemisphere. Modification in the endogenous metabolites concentrations were monitored using localized ¹H MRS in a voxel located in the mouse striatum. Dynamic measurements of lactate consumption were acquired following i.v. injection of HP L-[1-¹³C]lactate solution and ¹³C MR spectra were acquired every 3 s post injection in the entire brain. Endogenous lactate quantification from ¹H MRS showed a strong increase in the ischemic region compared to sham. Infusion of HP-L-[1-¹³C]lactate led to ¹³C labeling of pyruvate. Calculated pyruvate-to-lactate ratio showed an increased labeling of the pyruvate pool in MCAO when compared to sham. This study shows the feasibility of measuring lactate metabolism in vivo in a mouse model of stroke following intravenous injection of HP L-[1-¹³C]lactate, offering new perspectives to understand lactate biodistribution and its neuroprotective effect in stroke.

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22. Dysregulation of protein synthesis: a molecular link between FXS and non-syndromic ASD

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Autism Spectrum Disorders (ASDs) are a range of clinical conditions characterized by high phenotypic heterogeneity. There are several risk factors that may contribute to ASD which include genetic mutations and environmental factors. A few single-gene disorders are associated with autism: one of them is the Fragile X syndrome (FXS). The Fragile X Syndrome is caused by the unstable expansion of the CGG repeat in the 5'UTR region of the FMR1 gene, located on the X chromosome. As a consequence of this transcriptional silencing, the protein product (FMRP) of the FMR1 gene is absent. FXS and ASD share some clinical and molecular features, like aberrant spine morphology, altered synaptic function and behavior. Moreover they are characterized by the dysregulation of PI3K/Akt/mTOR and Ras/Raf/ERK 1-2 signaling pathways that lead to protein synthesis disruption. Recently our team has shown that a dysregulation of protein synthesis - observed in Fmr1 KO mice - is directly correlated with the excessive production of sAPP α , generated by the α -cleavage of APP by the secretase ADAM10 (Pasciuto et al., 2015 Neuron). sAPP α is also upregulated in children with severe autism and FXS. Given this evidence, we analyzed APP, ADAM10 and sAPP α levels in fibroblasts from FXS patients to validate in humans the dysregulation of APP metabolism observed in the Fmr1 KO mice. Furthermore we checked the disruption of protein synthesis levels in this cohort of patients. We also analysed the APP levels in idiopathic autism. In addition, we are currently investigating the hypothesis that dysregulation in the PI3K/Akt/mTOR and Ras/Raf /ERK 1/2 cascades might be affected in blood from patients with ASD. Our data on a small cohort of patients (n = 15) show that the molecular profiling in these patients indeed correlate with the severity degree of ASD.