4TH EUROPEAN SYNAPSE MEETING

AUGUST 28TH-30TH, 2013
BORDEAUX

TOPICS COVERED AND SPEAKERS

PRESYNAPTIC MECHANISMS

MATTHIJS VERHAGE, AMSTERDAM
TIM RYAN, NEW-YORK
ZOLTAN NUSSER, BUDAPEST
MIKE COUSIN, EDINBURGH
ERICH WANKER, BERLIN

MOLECULAR ARCHITECTURE OF SYNAPSES

STEPHEN SMITH, STANFORD
JEAN-LOUIS BESSEREAU, LYON
VLADAN LUCIC, MARTINSRIED
JEAN-PHILIPPE PIN, MONTPELLIER
RADU ARICESCU, OXFORD

TRAFFICKING AND SYNAPTOGENESIS

NATHALIE SANS, BORDEAUX
NICOLA ALLEN, SAN DIEGO
ARNIVAN GOSH, BASEL
PETER SCHEIFFELE, BASEL

SYNAPTIC PLASTICITY AND NETWORKS

THOMAS OERTNER, HAMBURG
MICHAEL HÄUSSER, LONDON
LAURE RONDI-REIG, PARIS
CHRISTIAN LUSCHER, GENEVA
SETH GRANT, EDINBURGH
JEFF MAGEE, ASHBURN

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4-6 TALKS
WILL BE SELECTED FROM ABSTRACTS

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ABSTRACT BOOK

Invited Speakers

S1. **Nicola ALLEN**, San Diego - *Astrocyte regulation of neuronal glutamate receptors*

S2. **Radu ARICESCU**, Oxford - *Mind the gap: trans-synaptic protein networks to learn and remember*

S3. **Jean-Louis BESSEREAU**, Lyon - *Extracellular scaffolds: an alternative perspective for post-synaptic organization*

S4. **Mike COUSIN**, Edinburgh - *Control of synaptobrevin retrieval by synaptophysin in health and disease*

S5. **Arnivan GOSH**, Basel – *Title missing*

S6. **Seth GRANT**, Edinburg - *Deconstructing synapse complexity*

S7. **Vladan LUCIC**, Martinsried - *Imaging neuronal synapses at molecular resolution by cryo-electron tomography*

S8. **Christian LÜSCHER**, Geneva - *Drug-evoked synaptic plasticity: from molecular mechanism to behavioural correlates and back*

S9. **Jeff MAGEE**, Ashburn - *The high spine neck resistance in CA1 pyramids increases nonlinear input interactions*

S10. **Zoltan NUSSER**, Budapest - *Quantitative molecular differences in presynaptic active zones*

S11. **Thomas OERTNER**, Hamburgh - *Form follows function: Reverse engineering of spine synapses*

S12. **Jean-Philippe PIN**, Montpellier - *Sensing mGlu receptor activation and modulation*

S13. **Laure RONDI-REIG**, Paris - *Interaction between cerebellum and hippocampus in sequence learning: anatomo-functional studies in human and mice*

S14. **Timothy RYAN**, New-York - *Interplay of Ca and K channels in controlling presynaptic waveforms, calcium influx and exocytosis*

S15. **Nathalie SANS**, Bordeaux - *Regulation of glutamate receptor trafficking by scaffolding proteins of the planar cell polarity signaling pathway*

S16. **Peter SCHEIFFELE**, Basel - *Deconvolving molecular diversity of synaptic adhesion molecules*

S17. **Stephen SMITH**, Stanford - *From brain maps to brain mechanisms: synapse molecular diversity*

S18. **Christophe SCHMIDT-HIEBER**, London - *Probing synaptic mechanisms of grid cell formation*

S19. **Matthijs VERHAGE**, Amsterdam - *Docking, priming and fusion of dense core vesicles in mammalian CNS neurons*

S20. **Erich WANKER**, Berlin - *First interactome maps for synaptic proteins*

Selected Short Talks

ST1. **Zafar BASHIR**, Bristol **-** *NMDA receptor-mediated metaplasticity at schaffer collateral-CA1 synapses*

ST2. **Thomas BIEDERER**, Yale **-** *Activity-driven changes in the synaptogenic potential of neurons modulate memory processing*

ST3. **Mario CARTA**, Bordeaux - *Membrane lipids tune synaptic transmission by direct modulation of potassium channels*

ST4. **Lydia DANGLOT**, Paris - *Role of the vesicular SNARE TI-VAMP in post-synaptic receptors distribution and traficking at hippocampal synapse*

ST5. **Fabrizio GARDONI**, Milan - *A novel partner for GluN2A-containing NMDA receptors, RNF10: a synapse-to-nucleus signal*

ST6. **Helmut KESSELS**, Amsterdam - *NMDA receptor dependent long-term synaptic depression is independent of ion flow through NMDA receptors*

ST7. **Elisabeth MENNA**, Milan - *The role of the actin capping protein Eps8 in synapse formation and function*

ST8. **Fekrije SELIMI**, Paris - *Two afferents, one target: what are the molecular pathways controlling their specific connectivity?*

ST9. **Katalin TOTH**, Quebec - *Distinct pool of vesicles contributing to asynchronous release are generated via bulk endocytosis*

ST10. **Jakob Von ENGELHARDT**, Heidelberg - *CKAMP44 and TARP- γ8 modulate gating and promote surface expression of AMPA receptors in dentate gyrus granule cells*

Abstracts Posters

P1. **Michael ACCARDI**, Montréal - *Mitochondrial reactive oxygen species regulate the strength of inhibitory neurotransmission by uncovering silent GABAergic synapses* - ***Cancelled registration***

P2. **Elisabetta ALOISI**, Bordeaux - *Surface dynamics of mGlu5 receptors in a mouse model of fragile X syndrome*

P3. **Paul BANKS**, Bristol - *Control of hippocampal to medial prefrontal cortex activity by long-term depression of NMDA receptor mediated transmission*

P4. **Camilla BELLONE**, Geneve - *Expression of cocaine-evoked synaptic plasticity by GluN3A-containing NMDA receptors*

P5. **Philipp BETHGE**, Bordeaux - *Two-photon excitation STED microscopy in two colors in acute brain slices*

P6. **Mehdi BHOURI**, Bristol - *NMDA receptor-mediated metaplasticity occurs selectively at Schaffer collateral-CA1 synapses*

P7. **Pierre BILLUART**, Paris - *Cognitive disorder genes and neuronal morphology characterization*

P8. **Andreas BJÖREFELDT**, Gothenburg - *The cerebrospinal fluid promotes excitatory neuronal activity*

P9. **Paolo BOTTA**, Basel - *Tonic inhibition in Central Amygdala controls anxiety*

P10. **Paola CACCIN**, Padova - *Effect of synaptic polybasic peptides on neurotransmission*

P11. **Ana Luisa CARVALHO**, Coimbra - *Modulation of hippocampal glutamatergic synapses by ghrelin*

P12. **Ronan CHÉREAU**, Bordeaux - *STED imaging of axon morphology and plasticity*

P13. **Amber CLAYTON**, Oxford - *Structural and biophysical analysis of proteins interacting with the extracellular region of AMPA receptor subunits*

P14. **Claude COLOMER**, Rotterdam - *Action potential regulation of Arc expression*

P15. **Sarah COUSINS**, London - *NETO-1 associates with the NMDA receptor/APP protein complex*

P16. **Patrizia D'ADAMO**, Milan - *The in vitro and in vivo characterization of RAB39B: an intellectual disability and autism spectrum disorder protein controlling the ER-exit of GluA2 AMPAR and cognitive functions*

P17. **Jary DELGADO**, Bordeaux - *Pin1-mediated phosphorylation-dependent prolyl isomerization regulates excitatory synapses via a PSD-95 N-terminus conformational change*

P18. **Julien DUPUIS**, Bordeaux - *Single molecule crosstalk between surface NMDA and dopamine D1 receptors tunes plasticity at hippocampal excitatory synapses*

P19. **Sabine FIÈVRE**, Bordeaux - *Molecular determinants for targeting of glutamate receptors at hippocampal mossy fiber synapses*

P20. **Maria FIUZA**, Pamplona - *GluN3A expression restricts spine maturation via inhibition of GIT1/Rac1 signaling*

P21. **Yoshiyuki FUKUDA**, Bayern - *Exploring synaptic architecture by cryo-electron tomography of FIB-milled neuronal cells*

P22. **Frederic GAMBINO**,Geneva - *Nonlinear paralemniscal thalamic inputs mediate whisker-evoked heterosynaptic plasticity in the somatosensory cortex*

P23. **Maurice GARRET**, Bordeaux - *Alteration of GABAergic neurotransmission within striatum and globus pallidus in R6/1 Huntington disease model*

P24. **Jimmy GEORGE**, Bordeaux - *Microglial Adenosine 2a receptor modulates MF-CA3 synaptic transmission via the release of extracellular ATP*

P25. **Adam GORLEWICZ**, Bordeaux - *The role of kainate receptors in acute models of epilepsy*

P26. **Laurie-Anne GOUTY-COLOMER**, Rotterdam - *Arc expression identifies the neuronal ensemble within the lateral amygdala recruited during fear conditioning*

P27. **Agata GOZDZ**, Warsaw - *GSK3a/b affect Arc protein expression in primary neuronal culture*

P28. **Marilena GRIGUOLI**, Bordeaux - *Cholinergic modulation of intact CA3 circuits in vivo*

P29. **Matthias HABERL**, Bordeaux - *Anterograde tracing using a novel envelope-switched ΔG rabies virus variant*

P30. **Etienne HERZOG**, Bordeaux - *Vesicular glutamate transporters as markers and players of synaptic vesicle mobility*

P31. **Julie JÉZÉQUEL**, Bordeaux - *Altered surface interplay between NMDA and dopamine receptors in a neuropsychiatric disorder*

P32. **Pooja JOSHI**, Milan - *Microglia convert aggregated amyloid-β into neurotoxic forms through the shedding of microvesicles*

P33. **Haruyuki KAMIYA**, Sapporo - *Photoinactivation analysis of synaptic AMPA receptor dynamics*

P34. **Rahul KAUSHIK**, Magdeburg - *The synapto-nuclear messenger Jacob directly associates with the CREB complex*

P35. **Christoph KOERBER**, Heidelberg - *Gene expression profiling of globular bushy cells during synap-tic maturation*

P36. **Remy KUSTERS**, Eindhoven - *Barriers in the brain: Receptor trafficking in dendritic spines*

P37. **Laurent LADÉPÊCHE**, Bordeaux - *Plasticity of maturing glutamate synapses requires NMDA receptors lateral mobility*

P38. **Capucine Le GUEN**, London - *Can neuromodulators affect electrical coupling between cerebellar Golgi cells?*

P39. **Flavie LESEPT**, Caen - *Subcellular trafficking of neuronal tPA and its potential role in synaptic plasticity*

P40. **Mathieu LETELLIER**, Bordeaux - *MicroRNA miR-92a regulates translation and synaptic incorporation of GluA1 containing AMPA receptors during homeostatic scaling*

P41. **Jolanta LUNDGREN**, Stockholm - *Comparing release mechanisms for glutamate and the amyloid?-peptide from rat brain nerve terminals*

P42. **Romain LY**, Paris - *T-type channel blockade impairs parallel fiber – purkinje cell long-term potentiation and cerebellar learning* - ***Cancelled registration***

P43. **Vincent MAINGRET**, Bordeaux - *PGE2 modulates synaptic plasticity at hippocampal mossy fiber - CA3 synapse*

P44. **Manuel MAMELI**, Paris - *Drug-evoked synaptic plasticity in the lateral habenula: a substrate for aversive states*

P45. **Wilfrid MAZIER**, San Juan de Alicante - *GluK1 overexpression in a mouse model of Down syndrome results in an increase of inhibitory synaptic transmission*

P46. **Zofia MIJAKOWSKA,** Warsaw - *αCaMKII-autophosphorylation in the development of addiction*

P47. **Nikolaos MITAKIDIS**, Oxford - *Proteoglycan regulation of RPTP?-TrkC mediated synaptogenesis*

P48. **Maïté MOREAU**, Bordeaux - *Short-term and long-term memory deficits in Looptail heterozytote mice mutated for the planar polarity protein Vangl2*

P49. **Edoardo MORETTO**, Milan - *TSPAN5 protein in the brain: a new regulator of GluA2 trafficking*

P50. **Stefano MUSARDO**, Milan - *ADAM10 exocytosis/endocytosis in spines: role in Alzheimer's disease*

P51. **Nael NADIF KASRI**, Nijmegen - *A critical role for ARHGAP12 in regulating excitatory synaptic structure and function*

P52. **Valentin NÄGERL**, Bordeaux - *Microglia-synapse interactions during synaptic plasticity*

P53. **Richard ORTEGA**, Gradignan - *Iron storage within dopamine vesicles revealed by synchrotron radiation X-ray fluorescence nano-imaging*

P54. **Aude PANATIER**, Bruges - *Nanoscale imaging of the tripartite synapse morphology using STED microscopy*

P55. **Andrew PENN**, Bordeaux – Cross-linking AMPA receptors and synaptic physiology in intact tissue

P56. **David PERRAIS**, Bordeaux - *Endocytosis and recycling of receptors in neuronal dendrites*

P57. **Bérangère PINAN-LUCARRE**, Villeurbanne - *An ADAMTS-Like protein organizes post-synaptic domains in C. elegans*

P58. **Vera PINHEIRO**, Bordeaux - *The autism associated Planar Cell Polarity protein Scribble1 regulates AMPA receptor-dependent synaptic plasticity trafficking*

P59. **Maria Joana PINTO**, Vila do Bispo - *Proteasome as a local regulator of presynapse assembly*

P60. **Joana REIS PEDRO**, Coimbra - *Intra-axonal translation of β-actin is required for presynaptogenesis*

P61. **Natalia H REVELO**, Göttingen - *Inner hair cells functionally separate synaptic vesicle recycling from constitutive membrane trafficking*

P62. **Andreas RITZAU-JOST**, Leipzig - *kHz transmission at a central synapse*

P63. **Morgane ROSENDALE**, Bordeaux - *Detecting the formation of endocytic vesicles in the soma and dendrites of live cultured neurons*

P64. **Laura RUSCHKIES**, Hamburg - *Conditional knockout and characterization of microtubule severing enzymes in mice*

P65. **Mariano SOIZA-REILLY**, Paris - *Developmental synaptic innervation of the mouse dorsal raphe nucleus using array tomography*

P66. **Jennifer STANIC**, Montreuil - *Rabphilin 3A interacts with GluN2A NMDA receptor subunit in the post-synaptic density*

P67. **Erica TAGLIATTI**, Genova - *phosphorylation of synapsin i by Cdk5 sets the ratio between the resting and recycling pools of synaptic vesicles at hippocampal synapses*

P68. **Tomi TAIRA**, Helsinki - *Synaptic kainate receptors in CA1 interneurons gate the threshold of theta frequency-induced long-term potentiation*

P69. **Agnes THALHAMMER**, Genova - *Compartmentalized expression of P/Q-type Ca2+ channel isoforms shapes neurotransmitter release and short-term synaptic plasticity at central synapses*

P70. **Cezar TIGARET**, Bristol - *Postsynaptic spine Ca2+ transients do not correlate with spike timing-dependent plasticity in mature CA1 hippocampal pyramidal neurons*

P71. **Marilyn TIRARD**, Goettingen - *Analysis of neuronal SUMOylation using TAP-SUMO knock-in mice*

P72. **Jan TØNNESEN**, Bordeaux - *Nanoscale spine neck morphology regulates compartmentalization of synapses*

P73. **Philipp TREPTE**, Berlin - *Generation of an interactome map for synaptic proteins*

P74. **Haijun TU**, Villeurbanne - *A Novel PUNCTIN/MADD-4 Pathway Required for GABA receptor clustering in Caenorhabditis elegans*

P75. Emilia TURCO, Torino **-** *p140Cap regulates memory and synaptic plasticity through Src- and CitN-mediated RhoA control*

P76. Silvia VIANA DA SILVA, Bordeaux **-** Synapse specific alterations in morphology and function of ca3 pyramidal cells in a mouse model of alzheimer's disease

P77. John WESSELING, Pamplona **-** *Parallel recruitment of slow and fast releasing vesicles to the RRP at Calyx of held synapses*

P78. Stefania ZAMBETTI, Milan **-** *The actin capping activity of Eps8 is required for spine morphogenesis and plasticity*

P79. Jonathan ZAPATA, Milan - *The X-LID protein SHROOM4 regulates GABAB receptors trafficking through its association with the microtubule-dependent molecular motor dynein*

P80. Benoît ZUBER, Bern **-** *Structure and superorganization of acetylcholine receptor-rapsyn complexes*

P81. Stefano ZUCCA, Bordeaux **-** *Analysis of synaptic plasticity in CA3 pyramidal cells in vivo using optogenetics tools*

Invited Speakers

S1. Nicola ALLEN

San Diego, USA
nallen@salk.edu

Astrocyte regulation of neuronal glutamate receptors

Salk Institute for Biological Studies, 10010 North Torrey Pines Rd, La Jolla, CA, 92037, USA

Astrocyte-secreted factors have been shown to powerfully induce the formation of functional excitatory synapses between CNS neurons. Astrocyte-secreted thrombospondins and hevin were identified as factors that induce structural synapses, however these synapses are post-synaptically silent due to a failure to recruit AMPA glutamate receptors (AMPAR) to the synapse. We identified glypicans, in particular glypicans 4 and 6, as a family of astrocyte-secreted factors that are sufficient to induce functional synapse formation between neurons and regulate neuronal AMPA receptor localisation. Application of glypican 4 to purified neurons is sufficient to increase the frequency and amplitude of glutamatergic synaptic events, and this is achieved by increasing the surface level and clustering, but not overall cellular protein level, of the GluA1 subunit of the AMPAR. Conversely, depletion of both glypican 4 and glypican 6 from astrocyte conditioned media (ACM) significantly reduces its ability to induce postsynaptic activity in neurons. In vivo expression analysis demonstrated that glypican 4 and 6 are expressed by astrocytes *in vivo* in the developing CNS, with glypican 4 expression enriched in the hippocampus and glypican 6 in the cerebellum. Glypican 4 deficient mice have defective synaptic function in the hippocampus during early postnatal development, shown by a decreased amplitude of excitatory synaptic currents and reduced recruitment of AMPARs to synapses. We are currently investigating the molecular mechanisms underlying the actions of glypicans, including identifying the neuronal receptor that mediates glypicans actions and the downstream signalling cascades activated in neurons upon glypican exposure.

S2. Radu ARICESCU, Oxford

Mind the gap: trans-synaptic protein networks to learn and remember

S3. Jean-Louis BESSEREAU

Lyon, France
jean-louis.bessereau@univ-lyon1.fr

Extracellular scaffolds: an alternative perspective for postsynaptic organization

B. Pinan-Lucarre[1,2], H. Tu[1, 2], M. Pierron[1,2], T. Ji[1] and Jean-Louis Bessereau[1, 2]
1. Institute of Biology of the Ecole Normale Superieure, Paris, France
2. University Claude Bernard, Lyon, France

Since most neurons receive thousands of synaptic inputs, the neuronal membrane is a mosaic of specialized microdomains where neurotransmitter receptors cluster in register with the corresponding presynaptic neurotransmitter release sites. The *Caenorhabditis elegans* neuromuscular junction (NMJ) provides a genetically tractable system to analyze the segregation of neurotransmitter receptors because muscle cells receive excitatory innervation from cholinergic neurons and inhibitory innervation from GABAergic neurons. Over the last years, we analyzed the mechanisms supporting the positioning of nicotinic acetylcholine receptors (AChRs) at NMJs. We uncovered a novel clustering system relying on an extracellular scaffold built in the synaptic cleft that involves the AChRs and at least 3 extracellular scaffolding proteins (Gally *et al.*, *Nature 2005*; Gendrel *et al.*, *Nature 2009*; Rapti *et al.*, *EMBO J.*, 2011). A novel genetic screen based on direct visualization of AChRs fluorescently-tagged by homologous recombination identified a mutant in which AChR and GABAR clusters were fragmented and redistributed on the muscle cell surface away from synapses. The mutated gene codes for an extracellular matrix protein from the ADAMTS-like family. This protein is secreted by motoneurons and localizes at NMJs. Alternative promoters generate different isoforms with distinct functions. The differential expression of these isoforms controls the congruence between pre- and post-

synaptic domains: specific disruption of the short isoform relocalizes GABARs from GABAergic to cholinergic synapses, while expression of a long isoform in GABAergic neurons recruits ACh receptors to GABAergic NMJs. These results identify a novel synaptic organizer and demonstrate that pre- and post-synaptic domain identities can be genetically uncoupled in vivo. Since the human ortholog of this ADAMTS-Like protein is expressed in the central nervous system and was identified as a candidate gene for schizophrenia, similar system might control synapse organization in the mammalian central nervous system.

S4. Mike COUSIN

Edinburgh, United Kingdom
M.Cousin@ed.ac.uk

Control of synaptobrevin retrieval by synaptophysin in health and disease

Sarah L. Gordon and Michael A. Cousin
Centre for Integrative Physiology, Hugh Robson Building, George Square, University of Edinburgh, Edinburgh, Scotland, UK.

Synaptophysin is an integral synaptic vesicle (SV) protein that accounts for approximately 10% of total SV protein cargo. Ablation of synaptophysin expression results no obvious defect in either neurotransmitter release or SV turnover, however knockout mice do display mild deficits in learning and memory. Recently we identified a specific defect in the retrieval of synaptobrevin II (sybII) from the plasma membrane during endocytosis in neurones cultured from synaptophysin knockout mice, in addition to a slowing in the general speed of SV endocytosis. Synaptophysin is implicated in X-linked intellectual disability (XLID), with a recent study identifying four separate gene mutations in families affected by the disorder. To determine how these mutations may impact on synaptophysin function, they were expressed in cultured neurons derived from synaptophysin knockout mice. Two distinct truncating mutants were mislocalised throughout the axon and failed to rescue sybII retrieval. Furthermore these mutants were dominant negative for sybII retrieval when expressed in wild-type neurones. The remaining two mutants displayed a nerve terminal localisation but again did not support efficient sybII retrieval from the plasma membrane. Out of these two mutants, one fully rescued SV endocytosis kinetics, suggesting the processes of sybII retrieval and endocytosis speed are independent from each other. These studies suggest that the efficient retrieval and sorting of sybII by synaptophysin may be key to maintaining synaptic health and perturbation of this event may contribute to the pathogenesis underlying neurodevelopmental disorders such as XLID.

S5. Arnivan GOSH, Basel

Title missing

S6. Seth GRANT

Edinburg, United Kingdom
seth.grant@ed.ac.uk

Deconstructing synapse complexity

The postsynaptic membrane of central neurons is the point of entry for information encoded in neural activity and signaling events within the postsynaptic proteome play a fundamental role in behaviors including reflexes, instincts, multiple forms of learning and other components of cognition. The protein machinery within the postsynaptic terminal has in the last decade been shown to be remarkably complicated with ~1500 proteins. Individual proteins not only assemble into complexes of several subunits, but these complexes can be building blocks for larger structures known as supercomplexes Although there are well described synaptic complexes comprising fewer than 4-5 subunits, such as SNARE complexes and ion channel/receptor complexes, with molecular weights <1MDa, very little is known about supercomplexes in the nervous system. We have developed tools for isolating and examining synaptic supercomplexes and will describe how they assemble, why they are important for diseases and behavior, and how they evolved.

S7. Vladan LUCIC

Martinsried, Germany
vladan@biochem.mpg.de

Imaging neuronal synapses at molecular resolution by cryo-electron tomography

The presynaptic terminal contains a complex network of filaments whose precise organization and functions are not yet understood. We have investigated this presynaptic cytomatrix by cryo-electron tomography, a method that allows visualization of vitrified, fully hydrated biological samples at molecular resolution. The wealth of information present in cryo-tomograms of synapses is a challenge for the interpretation of the visualized structures that we address by both biochemical and image processing methods.
We have previously shown that synaptic vesicles are embedded in a complex filamentous network and that prior to fusion, vesicles are tethered to the active zone membrane by short filaments. In order to gain insights into the identity of the molecules that form and regulate tethers, we analyzed presynaptic architecture of RIM1α knock-out (KO) mice by cryo-electron tomography of isolated synapses. In contrast to previous work on dehydrated, chemically-fixed samples, our data shows significant alterations in vesicle distribution and AZ tethering that could provide a structural basis for the functional deficits of RIM1α KO synapses. Proteasome inhibition reversed these structural defects, suggesting a functional recovery subsequently confirmed by electrophysiological recordings. Altogether, our results not only point to the ubiquitin-proteasome system as an important regulator of presynaptic architecture and function, but also show that the tethering machinery plays a critical role in exocytosis, converging into a structural model of synaptic vesicle priming by RIM1α.
Recent instrumentation development are promising to further improve the quality of cryo-tomograms. Better transfer properties and lower noise of direct high-energy electron detectors are already increasing the achievable resolution. Furthermore, focused ion beam thinning of vitrified samples allowed us to record cryo-tomograms of thicker samples, such as synapses of neurons grown in culture.

S8. Christian LÜSCHER

Geneva, Suisse
Christian.Luscher@unige.ch

Drug-evoked synaptic plasticity: from molecular mechanism to behavioural correlates and back

Cue-induced drug seeking as a major trigger for relapse in addiction. Here we parse the circuit adaptation underlying this behaviour. In line with prior literature, we focus on the convergence of several glutamatergic inputs onto neurons of the nucleus accumbens. We then characterise the drug-adpative changes at identified synapses, and with this insight design reversal strategies to normalise transmission. When applied in vivo, these optogenetic manipulations are capable of restoring baseline synaptic transmission and by this token erase cue-induced drug seeking. In summary our work proposes a causal relationship between specific forms of drug-evoked synaptic plasticity and core components of addiction related behaviour.

S9. Jeff MAGEE

Ashburn, USA
maeej@janelia.hhmi.org

Synaptic amplification by high impedance dendritic spines enhances input co-operativity

Dendritic spines are the nearly ubiquitous site of excitatory synaptic input onto neurons and as such are critically positioned to influence diverse aspects of neuronal signaling. Decades of theoretical studies have proposed that spines may function as highly effective and modifiable chemical and electrical compartments that regulate synaptic efficacy, integration, and plasticity. Experimental studies have confirmed activity-dependent structural dynamics and biochemical compartmentalization by spines. However, a longstanding debate remains over the influence of spines on the electrical aspects of synaptic transmission and dendritic operation. We measured the amplitude ratio (AR) of spine head to parent dendrite voltage across a range of dendritic compartments and calculated the associated R_{neck} for spines at apical trunk dendrites in hippocampal CA1 pyramidal neurons. We found that R_{neck} is large enough (~500 MΩ) to substantially amplify the spine head depolarization associated with a unitary synaptic input by ~1.5- to ~45-fold depending on parent dendritic impedance. A morphologically realistic compartmental model capable of reproducing the observed spatial

profile of AR indicates that spines provide a consistently high impedance input structure throughout the dendritic arbor. Finally, we demonstrate that the amplification produced by spines encourages electrical interaction among coactive inputs through an R_{neck}-dependent increase in spine head voltage-dependent conductance activation. We conclude that the electrical properties of spines promote nonlinear dendritic processing and associated forms of plasticity and storage, thus fundamentally enhancing the computational capabilities of neurons.

S10. Zoltan NUSSER

Budapest, Hungary
nusser@koki.hu

Quantitative molecular differences in presynaptic active zones

Zoltan Nusser
Laboratory of Cellular Neurophysiology, Institute of Experimental Medicine, Budapest, Hungary

Synapses of the CNS display large structural and functional diversity, the underlying mechanism of which is largely unknown. I will present our experiments obtained with two-photon imaging-based optical quantal analysis and correlated electron microscopic (EM) 3D reconstruction, showing that the probability of glutamate release (Pr) from local axon collaterals of hippocampal CA3 pyramidal cells (PCs) scales linearly with the size of the active zone (AZ). Combined *in vitro* two-photon Ca^{2+} imaging from presynaptic boutons and correlated EM reconstruction of the imaged boutons revealed that the total fluxed Ca^{2+} following an action potential is also proportional with the AZ area. SDS-digested freeze-fracture replica immunogold labeling of the presynaptic voltage-gated Ca^{2+} channel subunit Cav2.1 showed that this ion channel is confined to the AZ within the axon terminals and its density is uniform across AZs. It has been known that the Pr and short-term plasticity patterns of PC axon terminals contacting parvalbumin or mGluR1α immunopositive GABAergic interneurons also display great variability. I will also present our recent data demonstrating that the underlying mechanism might be a lower presynaptic Ca^{2+} channel density at the AZ of terminals contacting mGluR1α positive cells. Our results provide evidence for quantitative differences in the molecular composition of the presynaptic AZs of local axon terminals of CA3 PCs, with the consequence of allowing the fine tuning the release probability.

S11. Thomas OERTNER, Hamburgh

Form follows function: Reverse engineering of spine synapses

S12. Jean-Philippe PIN

Montpellier, France
jean-philippe.pin@igf.cnrs.fr

Sensing mGlu receptor activation and modulation

University of Montpellier, CNRS, INSERM, Institute of Functional Genomics, F-34094 Montpellier, France

Metabotropic glutamate (mGlu) receptors are key modulators of synaptic transmission, regulating both pre- and post-synaptic processes. These receptors are more complex proteins than any other GPCRs, being composed of two subunits, each composed of three main domains: a venus flytrap domain (VFT, where glutamate binds) connected to a heptahelical domain (7TM, where positive allosteric modulators (PAM) bind) through a cystein-rich domain (CRD). Whereas agonists act by stabilizing a closed conformation of the VFT, how VFT closure leads to G protein activation remains unclear, but likely results from allosteric transitions within such a multidomain protein complex. Using innovative cell surface labeling technologies allowing the measurement of FRET signals in a time-controlled manner, we recently illustrate how these complex proteins work to activate heterotrimeric G proteins. We show that a movement of the VFT leads to a relative movement of the 7TMs, and as a consequence, the activation of only one of these able to activate G proteins. Using orthogonal labeling of two distinct cell surface proteins, we also illustrate the ability of these receptors to form heterodimeric complexes, and demonstrate their specific functional and pharmacological properties. Taken together, these data illustrate the coordinated allosteric transitions that link agonist binding into the VFT

clef, to G protein activation in such dimeric GPCR complexes, and reveal a new possible level of complexicity for these receptors in vivo.

S13. Laure RONDI-REIG
Paris, France
laure.rondi@snv.jussieu.fr

Interaction between cerebellum and hippocampus in sequence learning: anatomo-functional studies in human and mice

Spatial navigation is a complex function that requires the integration of heterogeneous information to build a coherent representation of the external world and drive optimal goal-directed behavior. One of the key neural substrate enabling such representation is the hippocampus, which contains pyramidal cells described as place cells. Each place cell fires for a restricted region of the environment. Both external (visual, olfactory, auditory, and somatosensory) and self-motion (vestibular, proprioceptive and optic flow signals) cues control place cell firing. This multimodal integration suggests that a large network of cortical and subcortical structures interacts with the hippocampus for navigation. Determining the functional architecture of such a network is essential to our understanding of how the spatial code is generated. Our aim is to decipher the role of the cerebellum in this network.

Cerebellum is classically described as a structure involved in motor function. However, using L7PKCI transgenic mice, we demonstrated that a deficit in cerebellar long-term depression (LTD) leads to a dysfunction of the hippocampal place cells (Rochefort et al., 2011) as well as impaired goal-directed navigation abilities (Burguiere et al., 2010), while no motor deficit is detected. This navigation deficit is specifically observed when mice had to rely on self-motion information. Our findings thus revealed that during navigation, the cerebellum communicates with the hippocampus to shape the spatial code. They further suggest that the hippocampus-cerebellum interaction is particularly important in navigation conditions relying on self-motion cues. Our current investigation focuses on this functional interaction. For this purpose we have adopted a multi-disciplinary approach to identify the cerebral network involved in different learning stages of a complex navigational task.

On one hand, we used fMRI to examine the cerebellar contribution to spatial navigation in humans. Subjects were scanned while performing the starmaze navigation task, recently developed in virtual reality and which can be solved using either place or sequence-based strategies. Our findings suggest that place-based navigation is supported by coherent activity in left cerebellar lobule VIIA, right hippocampus and medial parietal cortex, while sequential egocentric navigation is supported by coherent activity in right lobule VIIA, left hippocampus and medial prefrontal cortex. The specific exploration of the structures involved in motor control reveals that they are different from those involved in navigation.

On the other hand, we trained mice to learn a path in a multi-intersection maze without environmental cues, and identified the structures underlying this learning by Fos imaging at two stages: exploration, through which information is gathered to build a spatial representation of the environment, and stabilization (over-training). Because this path learning requires distinguishing between different locations by the order in which they are met and associating to each location a particular turn, it meets spatio-temporal sequence learning characteristics. Cerebellar lobules VI, IX, X have significant Fos density increase only in the exploration phase whereas prefrontal and parietal cortices, dorsal CA1, CA3, and ventral CA1 of the hippocampus are structures with significant Fos density increase in both exploration and stabilization phase. Interestingly, the cerebellum shows correlated Fos densities with hippocampal regions in both exploration and stabilization phases.

In conclusion, these results highlight the prominent role of cerebellum in navigation, an ethologically important cognitive task, and specify the cortico-cerebellar circuits. The cerebellum and the hippocampus participate through correlated activities in exploration and in spatio-temporal sequence learning suggesting that the cerebellum belongs to an anatomo-functional pathway through which the mental representation is built and used for navigation.

S14. Timothy RYAN
New-York, USA
taryan@med.cornell.edu

Interplay of Ca and K channels in controlling presynaptic waveforms, calcium influx and exocytosis

The detailed kinetics and amplitude of presynaptic action potentials (APs) are critical functional parameters in driving synaptic transmission as they determine in part the opening of voltage-gated calcium channels and the temporal profile of the driving force for subsequent calcium entry. Given the non-linear relationship of calcium entry and exocytosis subtle changes in calcium entry can have profound impact on synaptic efficacy. Using novel optical tools that allow us to characterize presynaptic voltage waveforms we have discovered that there is a homeostatic-like relationship between calcium channel abundance and the width of the presynaptic AP. Increasing calcium channel abundance leads to significant narrowing of presynaptic AP waveforms. Conversely lowering calcium channel abundance broadens the AP waveform, while lowering potassium channel abundance decreases calcium channel abundance and calcium influx. Thus presynaptic calcium and potassium channels are cross-regulated in a homeostatic manner.

S15. Nathalie SANS
Bordeaux, France
nathalie.sans@inserm.fr

Regulation of glutamate receptor traffic by scaffolding planar cell polarity proteins

INSERM, Planar Cell Polarity Group, Neurocentre Magendie, Unite U862 Physiopathologie de la plasticité neuronale, U862, F-33000 Bordeaux, France.
Univ. Bordeaux, Neurocentre Magendie, Physiopathologie de la plasticité neuronale, U862, F-33000 Bordeaux, France.

The appropriate trafficking of glutamate receptors to synapses is crucial for basic synaptic function and synaptic plasticity. It is now accepted that NMDA receptors, just like AMPA receptors, internalise and are recycled at the plasma membrane but also exchange between synaptic and extrasynaptic pool; these NMDAR properties are also key in governing synaptic plasticity. Scribble1 is a PDZ protein required for synaptogenesis and synaptic plasticity that has been recently implicated in neurodevelopmental disorders in patients. I will present data showing that the Scribble1 protein level, which is activity-dependent, regulates the localisation of the NMDAR subunits, GluN2A and GluN2B, at the plasma membrane. In particular, Scribble1 prevents GluN2A subunits from undergoing lysosomal trafficking and degradation by increasing their recycling to the plasma membrane following NMDAR activation. I will also present new evidences showing that PCP signaling is implicated in the regulation of synaptic activity, and that it impacts on learning and memory. Together these data highlight the central role of PCP signaling in glutamate receptor trafficking and synaptic plasticity.

S16. Peter SCHEIFFELE
Basel, Switzerland
peter.scheiffele@unibas.ch

Deconvolving molecular diversity of synaptic adhesion molecules

Biozentrum of the University of Basel, Switzerland

The assembly of functional neuronal circuits during development relies on an intricate interplay of cellular interactions, molecular recognition signals, and neuronal activity-dependent processes. Over the past 20 years, families of neuronal cell surface receptors have been identified that may exhibit remarkable molecular diversity. This diversity has been hypothesized to underlie selective trans-cellular interactions and cell-type specific properties, essentially as a molecular code for aspects of neuronal identity. We will discuss recent studies uncovering the molecular coding power of synaptic adhesion molecules in the nervous system and mechanisms underlying the temporal and spatial control of isoform repertoires in neuronal populations.

S17. Christoph SCHMIDT-HEIBER

London, United Kingdom
c.schmidt-hieber@ucl.ac.uk

Probing synaptic mechanisms of grid cell formation

Neurons in the medial entorhinal cortex (MEC) exhibit a remarkable grid-like spatial pattern of spike rates that has been proposed to represent a neural code for path integration. How grid cell firing in MEC stellate cells arises from the combination of intrinsic conductances and synaptic input is not well understood. To address this question, we combine in vitro and in vivo experiments. Using two-photon glutamate uncaging in MEC stellate cells in slices from medial entorhinal cortex, we are examining how their dendritic excitability may contribute to shaping the input-output function during grid cell firing. In parallel, we are making whole-cell patch-clamp recordings in mice navigating in a virtual reality environment, in order to determine the membrane potential signature of stellate cells during firing field crossings. Together, these experiments are providing crucial information for a quantitative understanding of the cellular basis of spatial navigation, as well as essential constraints for grid cell models.

S18. Stephen SMITH

Stanford, USA
sjsmith@stanford.edu

From brain maps to brain mechanisms: synapse molecular diversity

Substantial progress is being made today in mapping both structure and activity of the brain’s synaptic circuitry. Unfortunately, there remain formidable challenges to relating structural and functional maps, or “connectomes”, in ways that reveal the mechanisms underlying synaptic circuit function. One of these challenges centers on the molecular and functional diversity of individual synapses. Synapses of the mammalian central nervous system are individually complex and deeply heterogeneous structures that mediate complex, diverse, plastic and strongly modulated signaling functions. Though the evidence for synapse diversity is compelling, its nature and rules are not well known. To understand circuit mechanisms of brain function, richer and more quantitative information on synapse molecular and functional diversity is needed. I'll discuss the importance of this challenge and describe some new methods now being developed to meet it.

S19. Matthijs VERHAGE

Amsterdam, The Netherlands
m.verhage@vu.nl

Docking, priming and fusion of dense core vesicles in mammalian CNS neurons

Center for Neurogenomics and Cognitive Research (CNCR), Vrije Universiteit and VU Medical Center, Amsterdam, The Netherlands

The regulated secretion of chemical signals in the brain occurs principally from two organelles, synaptic vesicles and dense core vesicles (DCVs). DCVs contain a diverse collection of cargo, including many neuropeptides that trigger a multitude of modulatory effects with quite robust impact, for instance on memory, mood, pain, appetite or social behavior. In addition, many other signals depend on DCVs, like trophic factors, but also signals that typically do not diffuse like guidance cues. Hence, it is beyond doubt that DCV signalling is a central factor in brain communication. However, many fundamental questions remain open on DCV trafficking and secretion. My lab has established new photonic approaches to quantitatively characterize DCV-trafficking and fusion of many cargo types in living mammalian CNS neurons with single vesicle resolution using dual reporter cargo constructs. We found that DCV secretion is quite different from other forms of regulated secretion. DCVs often do not stably dock before fusion, molecular factors essential for synaptic vesicle release are dispensable for DCV fusion and DCV fusion occur with different kinetics at different cellular locations. The priming proteins Munc13 and CAPS are central factors in regulating both the kinetics and the location of DCV fusion events.

S20. Erich E. WANKER
Berlin, Germany
ewanker@mdc-berlin.de

First interactome maps for synaptic proteins

Max Delbrueck Center for Molecular Medicine (MDC), Department of Neuroproteomics, Robert-Roessle-Straße 10, 13125 Berlin, Germany

Proper functioning of synapses relies on protein-protein interactions (PPIs) and the assembly of multiprotein complexes. Abnormal PPIs can lead to severe neurological disorders like schizophrenia, epilepsy or neurodegenerative diseases. Hence, the systematic analysis of synaptic PPIs will provide new insights in key synaptic processes as well as shed light on important disease mechanisms. We have utilized the LUMIER (LUminescence-based Mammalian IntERactome mapping) technology and different yeast-two-hybrid (Y2H) screening methods to identify PPIs connecting ~1,000 synaptic proteins. By integrating Y2H PPI data with known literature interactions, we recently generated a first synapse interactome map, which links 1,340 proteins via 16,722 PPIs. Importantly, we observed that in the network proteins could be separated according to their likely pre- or postsynaptic localization. Next, using a computational strategy we predicted the localization of ~190 uncharacterized proteins in the pre- or postsynaptic compartment, of which a fraction was validated by subcellular fractionations and immunofluorescence microscopy. Furthermore, using the available PPI network, we linked a total of 46 proteins to the presynaptic active zone as well as to membrane fusion processes and endocytosis. Finally, interaction maps for neurological disorders such as for Alzheimer's disease were generated and experimentally validated with cell-based assays. Thus, we suggest that our interactome map for synaptic proteins is a valuable resource for functional validation studies and the investigation of disease mechanisms in synaptopathies.

ST1. Zafar BASHIR

Bristol, United Kingdom
zafar.bashir@bristol.ac.uk

NMDA Receptor-mediated metaplasticity occurs selectively at schaffer collateral-CA1 synapses

Mehdi Bhouri[1]*, Paul A Farrow[2]*, Aneeta Motee, Xu Yan[3], Stephen M Fitzjohn[4], Zafar I Bashir[1]
1. MRC Centre for Synaptic Plasticity-school of Physiology and Pharmacology-University of Bristol-UK,
2. Synaptic Signaling and Neurodegeneration-German Cancer Research Centre - Heidelberg-Germany,
3. Neuroscience Center-University of Helsinki-FINLAND
4. Lilly UK-Surrey-UK

The hippocampus is a key structure of the brain for learning and memory processes. We know that two inputs to the CA1 region the Schaffer collaterals (SC) and the temporoammonic (TA) pathways have different role in these processes Indeed the SC-CA1 input is necessary for spatial memory encoding and short term spatial memory, whereas the TA-CA1 input is critical for long-term spatial memory consolidation and retrieval. N-methyl-D-aspartate receptors (NMDAR) are crucial for induction of synaptic plasticity and learning and memory processes, and are themselves subject to plasticity NMDAR-mediated plasticity can therefore change the threshold for induction of future synaptic plasticity and affect learning and memory. However, little is known about the effect of NMDAR-mediated plasticity on subsequent long-term potentiation (LTP) and long-term depression (LTD). Using a combination of whole-cell recordings and field recordings in the CA1 area of the hippocampus, we have shown that the SC-CA1 input is subject to metaplasticity whereas the TA-CA1 input is not. Indeed a 5Hz 20s protocol induces LTD of NMDA receptor-mediated transmission in the SC-CA1 input but not in the TA-CA1 input. This LTD is mGlu receptor-dependent as it is blocked by application of LY311495, a group I mGluR antagonist. We have also shown that this LTD of NMDAR-mediated transmission prevents induction of LTP of AMPA transmission in the SC-CA1 input but not in the TA-CA1 input. However, LTD of AMPA transmission in both inputs is not affected by the LTD of NMDAR-mediated transmission. Therefore, these results show that the SC-CA1 input can undergo metaplasticity whereas the TA-CA1 input does not. These differences could explain the distinct roles of the two inputs in memory.

ST2. Thomas BIEDERER

Yale, USA
thomas.biederer@yale.edu

Activity-driven changes in the synaptogenic potential of neurons modulate memory processing

Rachel Jeffrey, Michael Honsberger, Jane Taylor, and Thomas Biederer
Department of Molecular Biophysics and Biochemistry, and Department of Psychiatry, Yale University

Synaptic cell adhesion molecules, including immunoglobulin proteins of the SynCAM family, have instructive roles in synapse development. The extent to which these trans-synaptic interactions modulate neuronal connectivity in the adult brain, however, remains an important knowledge gap. We observed that neuronal activation during learning is differentially regulated in SynCAM 1 overexpressor and knock-out mice. Further, memory acquisition results in a transient increase of SynCAM 1 amounts in synaptic fractions prepared from hippocampus. Aiming to gain insights into the activity-dependent roles of synaptic adhesion in the remodeling of synaptic connectivity, we developed a mouse model in which the neuronal activation that occurs naturally during behavior drives transgenic expression of SynCAM 1. This is the first model allowing for the select overexpression of a synapse-organizing protein in an activity-dependent manner and with temporal control. Activation of neurons in vivo was performed through fear conditioning, a robust behavioral paradigm that tests memory acquisition and processing. Importantly, select manipulation of SynCAM 1 levels only in the small subset of neurons active during memory acquisition yielded memory enhancements. Specifically, we found that transient SynCAM 1 overexpression during memory acquisition is linked to an increase in long-term memory performance. A systematic examination of distinct stages of memory acquisition determined that memory consolidation and extinction are also modulated by changes in synaptogenic potential. Further, our evidence supports that the transient overexpression of SynCAM 1 in select neurons results in their re-activation when a new task is learned, indicating that they get recruited into a memory trace. Concurrent analyses probe changes

in the molecular make-up of synapses tagged with activity-induced SynCAM 1 and complement studies in neuronal culture. Together, these studies aim to elucidate how the trans-synaptic interactions of a subset of neurons activated during behavioral tasks can instruct connectivity changes from a synaptic to a network level.

ST3. Mario CARTA

Bordeaux, France
mario.carta@u-bordeaux2.fr

Membrane lipids tune synaptic transmission by direct modulation of potassium channels

Mario Carta[1], Frederic Lanore[1], Nelson Rebola[1], Zsolt Szabo[1], Joana Lourenço[1], Silvia Viana da Silva[1], André Nadler[2], Carsten Schultz[2], Christophe Blanchet[1], Christophe Mulle[1]

1. University of Bordeaux, Interdisciplinary Institute for Neuroscience, UMR 5297, F-33000 Bordeaux, France;
2. EMBL Heidelberg, Meyerhofstraße 1, 69117 Heidelberg, Germany

Voltage-gated potassium (Kv) channels are involved in action potential repolarization in excitable cells. Exogenous application of membrane-derived lipids, such as arachidonic acid (AA), regulates the gating of Kv channels. Whether membrane-derived lipids released under physiological conditions impact on neuronal coding through this mechanism is unknown. We have investigated this possibility at mossy fiber to CA3 synapse in the hippocampus, where Kv channels in the presyantic terminal have been shown to modulate glutamate release. He have found that that AA released in an activity-dependent manner from postsynaptic hippocampal CA3 pyramidal cells acts as a retrograde messenger which induces robust facilitation of mossy fiber synaptic transmission. AA acts by broadening presynaptic action potentials through the direct modulation of Kv channels. This form of short-term plasticity can be triggered by natural patterns of spike discharge in the postsynaptic cell, and sets the threshold for the induction of the presynaptic form of long-term potentiation (LTP) at hippocampal mossy fiber synapses. Hence, direct modulation of presynaptic Kv channels by activity-dependent release of lipids serves as a physiological mechanism for the tuning of synaptic transmission.

ST4. Lydia DANGLOT

Paris, France
lydia.danglot@inserm.fr

Role of the vesicular SNARE TI-VAMP in post-synaptic receptors distribution and traficking at hippocampal synapse

Agathe Verraes[1], Olivier Pascual[2], Thomas Freret[3], Orestis Faklaris[1], Diana Molino[1], Sebastien Nola[1], Marteen Loos[4], Matthijs Verhage[4], Philippe Rostaing[5], Antoine Triller[5], Michel Boulouard[3], Lydia Danglot[1]*, and Thierry Galli[1]*

1. Institut Jacques Monod, Paris
2. Oncoflam - Lyon
3. GMPC, Caen
4. CNCR, Amsterdam
5. IBENS, Paris

During development and plasticity, synaptic molecules are transported to the synapse via vesicular and endosomal carriers. Fusion of these carriers with the synaptic zone, is achieved by SNARE proteins. We previously studied the role of two vesicular SNARE on presynaptic release of neurotransmitters and showed that Synaptobrevin 2 and TI-VAMP co-exist in hippocampal mossy fibers presynaptic terminals (PNAS 2006). Whereas Synaptobrevin mediates synchronous neurotransmitter release, we showed using the AP-3 mutant mice, that the lack of TI-VAMP in mossy fiber presynaptic terminal was correlated with the loss of an asynchronous release and changes in spontaneous release, indicating that Synaptobrevin and TI-VAMP provide different molecular mechanisms for release at presynaptic sites. Since, we have recently established knocked out mice for TI-VAMP (Danglot et al., J Neurosci, 2012) which are viable in contrast to Synaptobrevin KO. TI-VAMP Knockout mice display decreased brain weight and increased ventricle volume as measured by MRI. Actually, detailed behavioral characterization unraveled that TI-VAMP knockout was associated with increased anxiety, which suggest a role for TI-VAMP in higher brain functions. We are now planning to unravel the specificity of this two neuronal v-SNAREs at both pre and post-synaptic sites. Indeed, these two SNAREs are also expressed in post-synaptic compartments which constitute a place of intense trafficking. However, the

membrane compartments and the molecular mechanisms involved are still poorly defined. In epithelial cells, we previously showed that TI-VAMP depletion reduces the cell surface amount of tetraspanins microdomains which control EGFR diffusion and signling at the plasma membrane. Depletion of TI-VAMP or tetraspanin CD82 restrains EGFR diffusion at the cell surface as observed by Quantum dots video-microscopy. This is correlated with an increase recruitment of endocytic machinery and impaired MAPK signaling. Such a mechanism could also operate at the post-synaptic plasma membrane and have a profound impact on the physiology of the synapse. We are now unraveling the role of vesicular transport mediated by these 2 SNAREs (synaptobrevin 2 and TI-VAMP), in the dynamics of post-synaptic glutamate receptors during synaptogenesis and plasticity using KO mice. Our unpublished results indicate altered synaptic morphology both in hippocampal cultures and in situ. These defects are also correlated with modification of expression and targeting of glutamate post-synaptic receptors. The molecular mechanism by which vSNARE are controling glutamate receptor distribution in dendritic spine and PSD is investigated by super resolution microscopy and biochemical approaches. Moreover, synaptic plasticity is now under investigation with both electrophysiological recordings (LTP) and behavioral explorations concerning spatial and recognition memory.

ST5. Fabrizio GARDONI

Milan, Italy
fabrizio.gardoni@unimi.it

A novel partner for GluN2A-containing NMDA receptors, RNF10: a synapse-to-nucleus signal

Gardoni F, Dinamarca MC, Guzzetti F, Lim D, Caldarelli A, Genazzani AA, Stanic J, Di Luca M
Dept. Pharmacological and Biomolecular Sciences, University of Milano, Italy. DiSCAFF, Università degli Studi del Piemonte Orientale Amedeo Avogadro, Novara, Italy.

Among the cellular mechanisms required for modifications of dendritic spines, synapse-to-nucleus communication plays a key role in the regulation of the long-term structural changes. Emerging evidence indicates that multiple signalling pathways arising from dendritic spines converge to the nucleus regulating the expression of genes associated with changes of synapto-dendritic inputs. In the last decade, few synapto-nuclear protein messengers have been identified, and shown to play key roles in plasticity and synapse function. We recently identified Ring Finger Protein 10 (RNF10) as a new synapse-to-nucleus molecule, which responds to specific calcium signals at the postsynaptic compartment to elicit discrete changes at the transcriptional level. RNF10 is highly enriched at the excitatory synapse where it is associated to the GluN2A subunit of NMDA receptors. RNF10 is also present in the nucleus, where it is known to associate with Mesenchyme Homeobox 2 (Meox-2) transcription factor. Activation of synaptic NMDA receptors leads to RNF10 translocation from dendritic spines to the nucleus and induction of the expression of RNF10 target genes. Interestingly, modulation of RNF10 expression levels plays a fundamental role in regulating excitatory spine morphology under resting conditions as well as following activity-dependent synaptic plasticity.

ST6. Helmut KESSELS

Amsterdam, Netherlands
h.kessels@nin.knaw.nl

NMDA Receptor dependent long-term synaptic depression is independent of ion flow through NMDA receptors

Helmut Kessels, Sadegh Nabavi, Roberto Malinow
Universtity of California San Diego, Netherlands Institute For Neuroscience

NMDA receptor (NMDAR) activation controls long-term potentiation (LTP) as well as long- term depression (LTD) of synaptic transmission, cellular models of learning and memory. A long- standing view proposes that a high level of Ca2+ entry through NMDARs triggers LTP; lower Ca2+ entry triggers LTD. We show that ligand binding to NMDARs is sufficient to induce LTD; neither ion flow through NMDARs nor Ca2+ rise is required. However, basal levels of Ca2+ are permissively required. Lowering, but not maintaining, basal Ca2+ levels with Ca2+-chelators blocks LTD and drives strong synaptic potentiation, indicating that basal Ca2+ levels control NMDAR dependent LTD and basal synaptic transmission. Our findings indicate that NMDAR dependent LTD can weaken active synapses without raising postsynaptic calcium, thereby revising and expanding the mechanisms

controlling synaptic plasticity. A similar role for NMDARs underlies synaptic depression produced by beta amyloid, suggesting this novel mechanism may participate in pathophysiological processes.

ST7. Elisabeth MENNA

Milan, Italy
menna@in.cnr.it

The role of the actin capping protein Eps8 in synapse formation and function

The establishment of synaptic contacts between appropriate neurons is the basis for the formation of neural networks. Neuronal filopodia play an active role in synaptogenesis, and subsequently switch to more stable structures, the dendritic spines. There is a positive correlation between spine shape and dimensions and synaptic strength; also, abnormalities in spine number and morphology have been observed in a number of neurological disorders thus linking spine morphogenesis with plasticity processes eventually leading to memory formation. The process of spine formation and enlargement of spine head is tightly controlled by actin and by a number of acting regulating proteins working in concert. Among them Eps8, which is endowed with capping activity, is recruited to the spine head during long term potentiation and inhibition of its actin capping activity impairs spine enlargement and plasticity. Accordingly, mice lacking Eps8 display immature spines, which are unable to undergo potentiation, and are impaired in cognitive functions. Additionally, we found that reduction in the levels of Eps8 occurs in brains of patients affected by autism compared to controls. Our data reveal the key role of Eps8 actin capping activity in spine morphogenesis and plasticity and indicate that reductions in actin capping proteins may characterize forms of intellectual disabilities associated with spine defects.

ST8. Selimi FEKRIJE

Paris, France
fekrije.selimi@college-de-france.fr

Two afferents, one target: what are the molecular pathways controlling their specific connectivity?

Alessia Usardi[1], Séverine M. Sigoillot[1], Keerthana Iyer[1], Maëva Talleur[1], Joseph P. Doyle[2], Nathaniel Heintz[2], Fekrije Selimi[1]

1. Team MMSF, CIRB, Collège de France, 75005 Paris, France

2. Lab. For Molecular Biology, The Rockefeller University, USA

The mammalian brain is composed of many different types of neurons that form functional networks by establishing specific synapses. Any defects in the mechanisms leading to this precise connectivity can lead to neurodevelopmental disorders, as illustrated by the association of an increasing number of synaptic genes with diseases like autism or schizophrenia. Our goal is to understand the molecular mechanisms controlling the specific connectivity of different afferents on one target neuronal population. We use two approaches that allow us to "dissect molecularly“ the mouse brain in specific neurons and synapses: the synaptic protein profiling approach and the bacTRAP strategy. As a model, we are using the olivo-cerebellar network where Purkinje cells receive two excitatory glutamatergic inputs, one from cerebellar granule cells and one from inferior olivary neurons, which are distinct in terms of their physiology and of their innervating territory on Purkinje cells. We have compared the gene expression profiles of these two inputs using the bacTRAP technology and have found that the differentially expressed genes code for different functional categories of proteins. Inferior olivary neurons express an increased variety and level of membrane proteins compared to granule cells, in which the biggest cluster of differentially expressed genes code for nuclear proteins. This result can be correlated to the fundamental differences in terms of connectivity and morphology between these two neuronal types. Our comparison also informs us about the differentially expressed genes coding for secreted and membrane proteins that could be directly involved in the specific connectivity of these two inputs on Purkinje cells. For example different members of the complement family are expressed in the system: C1QL1 is expressed in inferior olivary neurons whereas CBLN1 and CBLN3 are expressed in granule cells. C1QL1 can interact with the Brain Angiogenesis Inhibitor receptor BAI3. Results from our laboratory show that the BAI3 receptor coordinates dendritogenesis and synaptogenesis in cerebellar Purkinje cells. Taken together, our results suggest a model involving C1QL1 proteins and BAI receptors in determining the territory of each input and the regulation of the proper balance of connectivity in the mammalian brain.

ST9. Katalin TOTH

Quebec, Canada
toth.katalin@crulrg.ulaval.ca

Distinct Pool of Vesicles Contributing To Asynchronous Release Are Generated Via Bulk Endocytosis

Alesya Evstratova[1], Victor Faundez[2], Katalin Toth[1]
1. Université de Laval, Quebec Mental Health Institute, Quebec City, Canada
2. Emory University, Atlanta, USA

Readily releasable pool of synaptic vesicles is composed of vesicles with heterogeneous molecular and functional properties. Emerging evidence suggest that three modes of neurotransmitter release: synchronous, asynchronous and spontaneous utilize distinct release machineries and might preferentially involve vesicles with specific properties. Functionally heterogenous vesicles in the same presynaptic terminal are generated via different endocytotic pathways. Clathrin-dependent endocytosis utilizes adaptor protein AP-2 while bulk endocytosis leads to the transient formation of endosomes and it is dependent on adaptor protein AP-3. The exact role of vesicles derived through the two recycling pathways in various forms of neurotransmitter release remains largely unknown. We used AP-3 knockout mice (Ap3b2-/-) lacking bulk endosomal vesicle formation to explore the physiological role of the vesicular pool formed via this pathway in synapses between mossy fibre boutons and hippocampal CA3 pyramidal cells. Synchronous release was tested at the range of physiological frequencies (0.1 – 20 Hz) and was similar in WT and KO mice. In contrast, asynchronous release was almost twice lower in KO compared to WT (3.4 ± 0.39 Hz and 7.8 ± 0.6 Hz, respectively). In both types of animals asynchronous EPSCs had higher amplitude and faster kinetic properties compared to spontaneous EPSCs. Application of the slow calcium chelator EGTA-AM (100 μM) significantly reduced the frequency of asynchronous events to 2.8 ± 1 Hz and 1.9 ± 0.5 Hz in WT and KO, respectively. We used deconvolution analysis to determine the rate of vesicle release during trains of stimulations. While there was no difference in the synchronous vesicle release rate, asynchronous release rate was significantly reduced already after the 3rd stimuli in the train (7.6 ± 1.4 vs 3.1 ± 0.8, for WT and KO respectively). Quantal parameters (p, Q and N), the size of the readily releasable pool and its recovery speed were comparable between WT and KO mice further underlining similar properties of synchronous neurotransmitter release. We aimed to determine the consequences of diminished asynchronous release on synaptic information transfer. Towards this aim we stimulated mossy fibres with natural spike trains recorded from behaving animals, while we recorded postsynaptic CA3 pyramidal cells in current clamp configuration. We analyzed the relationship between the presynaptic stimulus pattern and postsynaptic action potential (AP) generation. In the absence of asynchronous release the probability of postsynaptic AP generation during natural spike trains was reduced and the variability of the postsynaptic responses was increased. These findings indicate that during asynchronous release a distinct pool of vesicles generated via bulk endocytosis is released. Asynchronous release plays a crucial role in information coding, it is essential for optimal and reliable synaptic information transfer.

ST10. Jakob Von ENGELHARDT

Heidelberg, Germany
engelhardt@dzne.de

CKAMP44 and TARP-8 modulate gating and promote surface expression of ampa receptors in dentate gyrus granule cells

Jakob V Engelhardt, Eric Jacobi, Paul Farrow.
Synaptic Signalling and Neurodegeneration A300 DZNE DKFZ, Heidelberg, Germany

Trafficking, subcellular localization and gating of AMPA receptor depend on their interaction with auxiliary proteins such as TARPs, SynDig1, cornichons and CKAMP44. To investigate why some neurons express several auxiliary proteins with partially overlapping functions we used single and double-knockout mice of the auxiliary proteins CKAMP44 and TARP-γ8. We analyzed AMPAR-mediated currents in dentate gyrus granule cells, which display high expression levels of CKAMP44 and TARP-γ8. Both proteins increase apparent glutamate affinity and conductance and slow deactivation of AMPA receptors. However, the function of the two auxiliary proteins is not redundant since they exert an opposite influence on receptor desensitization. AMPA receptors that interact with TARP-γ8 and CKAMP44 exhibit a faster and slower recovery from desensitization, respectively, which explains the different influence of these auxiliary proteins on synaptic short-term plasticity. TARP-γ8 and

CKAMP44 not only influence gating but also promote surface expression of AMPA receptors. In fact, extrasynaptic and synaptic AMPA receptor-mediated current amplitudes are reduced in double-knockout mice to 5% and ca. 10%, respectively, showing that in dentate gyrus granule cells TARP-γ8 and CKAMP44 are the main auxiliary proteins that traffic AMPA receptors to the cell surface. In addition, these results strengthen the hypothesis that without auxiliary proteins AMPA receptors traffic very inefficiently to the cell surface.

Abstracts Posters

P1. Michael ACCARDI - *Cancelled registration*
Montreal, Quebec Canada
michael.accardi@mail.mcgill.ca

Mitochondrial reactive oxygen species regulate the strength of inhibitory neurotransmission by uncovering silent GABAergic synapses

Michael V Accardi, Shiva K Tyagarajan, Jean-Marc Fritschy, Derek Bowie.
McGill University, University of Zurich

Reactive oxygen species (ROS) are ubiquitous signaling molecules in the CNS primarily formed as by-products from mitochondrial metabolism. During periods of increased metabolism, mitochondrial ROS (mROS) generation is up-regulated. This is of particular physiological relevance in the context of synaptic function where elevated ROS levels may modulate synaptic neurotransmission. As the major inhibitory neurotransmission system in the brain, the GABAergic system has developed the sensitivity to react to constantly changing neuronal environments. Accordingly, we set out to uncover the influence of mitochondrial metabolism on the GABAergic system. Using patch-clamp recordings and mitochondrial poisons to elevate mROS, we observed a rapid increase in cerebellar interneuron GABAergic small amplitude, slow decaying miniature inhibitory postsynaptic currents (mIPSCs). The significance of mitochondrial stress in modulating GABAergic transmission was confirmed using the Fenton reaction and/or an antioxidant. Finally, in an attempt to elucidate the molecular basis for observed changes in GABAergic mIPSCs we used mutant α1- and α3-KO mice and identified the importance of α3-containing GABA-A receptors for this phenomenon. Taken together, our data identify a novel regulatory mechanism in which GABAergic transmission is modulated in a subunit-specific manner under the control of mROS. Our data suggests functionally active neurons may possess fast-acting compensatory mechanisms to down-regulate neuronal excitability by enhancing inhibition during periods of increased metabolic activity.

P2. Elisabetta ALOISI
Bordeaux, France
elisabetta.aloisi@inserm.fr

Surface dynamics of mGlu5 receptors in a mouse model of fragile X syndrome

Elisabetta Aloisi[1,2], Michela Spatuzza[3], Simona D'Antoni[3], Filippo Drago[4], *Maria Vincenza Catania[3], and *Andreas Frick[1]
1. International PhD program in Neuropharmacology, University of Catania, School of Medicine Catania, Italy
2. Neurocentre Magendie, INSERM U862, University Bordeaux 2, Bordeaux, France
3. Institute of Neurological Sciences, National Research Council (CNR), Catania, Italy
4. Dept. of Clinical and Molecular Biomedicine, University of Catania, School of Medicine Catania, Italy
** Equal contribution*

Altered metabotropic glutamate receptor subunit 5 (mGluR5) function is strongly implicated in the pathophysiology of Fragile X Syndrome (FXS), a leading inherited cause of intellectual disability and autism. The precise mechanisms underlying the defective mGluR5 signalling are not known and despite the prominent role of mGluR5 in the regulation of synaptic plasticity and cognitive functions very little is known about its trafficking and dynamics at the synapse. Previously, we have shown that in the animal model of FXS, the Fmr1 knockout (Fmr1 KO) mouse, mGluR5 is less associated with Homer proteins, which are post-synaptic density (PSD) partners of mGluR5. In this study we asked now the question what the consequence of this disrupted mGluR5/Homer crosslink is for the surface expression and spine membrane trafficking of these receptors. To achieve this we used a powerful combination of novel live-cell single molecule tracking together with immunocytochemical and biotinylation approaches in cultured hippocampal neurons from Fmr1 KO and littermate wild-type (WT) control mice. We show that the lateral mobility of mGluR5 is increased within the synapse but not at the extrasynaptic sites in neurons of Fmr1 KO mice as compared to WT mice. In agreement

with our prediction, disrupting the mGluR5/Homer crosslink in WT mice with a specific peptide mimics the Fmr1 KO phenotype by inducing a similar rate of mGluR5 surface diffusion. Despite their increased mobility in Fmr1 KO neurons, mGluR5s seem to be spatially more confined in their exploratory behaviour. This could be due to a steric hindrance within the membrane related to an excessive mGluR5-dependent protein synthesis and thus increased expression of membrane proteins in the disease state. Pre-treatment with the mGluR5 antagonist 2-methyl-6-(phenylethynyl)pyridine (i.e. MPEP) indeed rescues this phenotype to almost WT level. In summary, our findings demonstrate an alteration of the mGluR5 dynamics in the synaptic membrane of Fmr1 KO hippocampal neurons and provide a new cellular mechanism for the mGluR5 dependent pathophysiology in FXS.

P3. Paul BANKS
Bristol, United Kingdom
paul.banks@bristol.ac.uk

Control of hippocampal to medial prefrontal cortex activity by long-term depression of NMDA receptor mediated transmission

Paul Banks, Amelia Burroughs, Zafar Bashir
University of Bristol, Department of Physiology and Pharmacology, Bristol, UK

The hippocampus is connected to the medial prefrontal cortex (mPFC) by a long-range monosynaptic glutamatergic projection which is known to undergo activity-dependent modification. This connection has been shown to be involved in numerous cognitive processes including learning and memory. It has been shown in the mouse that by making a modified coronal slice of mPFC it is possible to stimulate a fibre bundle originating from the hippocampus using conventional electrophysiological techniques. We have now demonstrated that this slice preparation also works in the rat brain and studied plasticity of this pathway, focussing particularly on NMDAR transmission. We show that activation of G-protein coupled receptors could induce long-term depression (LTD) of pharmacologically isolated NMDAR-mediated transmission in the hippocampal-prefrontal pathway: bath application of group I mGluR agonist DHPG potently reduced NMDAR transmission for at least 1 hour. Similar effects were observed following application of the cholinergic agonist carbachol and mAChR1 agonist AF102B. Interestingly, we discovered that delivery of 300 stimuli at 5 Hz induces LTD selectively of NMDAR, but not of AMPAR mediated transmission. However, this activity-dependent LTD was not blocked by antagonists of muscarinic (scopolamine, atropine) or mGlu receptors (LY341495, MPEP, LY367385). Inhibition of NMDA receptors during the 5 Hz stimulation (by hyperpolarisation to -100 mV or by temporary wash in of AP5) also failed to block induction of NMDAR-LTD. Given the prominent role of dopamine in synaptic plasticity and learning and memory in mPFC we then investigated whether this transmitter may be involved in activity-dependent NMDAR-LTD. Interestingly we show that the antagonism of D2-like receptors (sulpiride), but not D1-like receptors (SCH23390) blocked induction of LTD. NMDARs have been shown to play a highly significant role in the temporal summation of bursts of synaptic stimuli. We demonstrated that this is also the case in the hippocampal-mPFC connection: pharmacological blockade of NMDARs considerably reduced summation of synaptic stimuli in a frequency dependent manner, with maximal effects at 20 and 50 Hz. We then showed that induction of NMDAR-LTD using 5 Hz stimulation also reduces temporal summation of synaptic stimuli in the same frequency dependent-manner, demonstrating that LTD of NMDAR transmission can have a marked effect on transmission from hippocampus to mPFC. These results demonstrate that activity-dependent plasticity of NMDAR transmission can markedly alter input-output characteristics of the hippocampal-mPFC pathway in a frequency-dependent manner - this may be important for learning and memory and other cognitive processes in which this synapse is involved.

P4. Camilla BELLONE
Genève, Switzerland
camilla.bellone@unige.ch

Expression of cocaine-evoked synaptic plasticity by GluN3A-containing NMDA receptors

Tifei Yuan, Manuel Mameli, Eoin C O'Connor, Partha Narayan Dey, Chiara Verpelli, Carlo Sala, Isabel Perez-Otano, Christian Lüscher, and Camilla Bellone
Department of Basic Neurosciences Medical Faculty, University of Geneva, CH-1211 Geneva, Switzerland

Drug-evoked synaptic plasticity in the mesolimbic dopamine (DA) system reorganizes neural circuits that may lead to compulsive behavior in addiction. The first cocaine exposure potentiates AMPAR excitatory postsynaptic currents (EPSCs) onto DA neurons of the VTA, but reduces the amplitude of NMDAR-EPSCs. While the plasticity of AMPAR transmission is expressed by insertion of calcium (Ca2+)-permeable GluA2-lacking receptors, little is known about the expression mechanism for the altered NMDAR transmission. Combining ex vivo patch clamp recordings, mouse genetics and subcellular Ca2+ imaging, we observe that cocaine drives the insertion of NMDARs that are quasi Ca2+-impermeable and contain GluN3A and GluN2B subunits. These GluN3A-containing NMDARs appear necessary for the expression of cocaine-evoked plasticity of AMPARs. We identify an mGluR1 dependent mechanism to remove these "non-canonical" NMDARs that requires Homer/Shank interaction and protein synthesis. Our data provide insight into the early cocaine-driven reorganization of glutamatergic transmission onto DA neurons and offers GluN3A-containing NMDARs as a new target in drug addiction.

P5. Philipp BETHGE

Bordeaux, France
philipp.bethge@u-bordeaux2.fr

Two-photon excitation STED microscopy in two colors in acute brain slices

Philipp Bethge[1,2], Ronan Chereau[1,2], Elena Avignone[1,2], Giovanni Marsicano[3], and U Valentin Nägerl[1,2].
1. Univ. Bordeaux, Interdisciplinary Institute For Neuroscience, UMR 5297, F-33000 Bordeaux, France
2. CNRS, Interdisciplinary Institute For Neuroscience, UMR 5297, F-33000 Bordeaux, France
3. Univ. Bordeaux, INSERM U862, NeuroCentre Magendie, F-33000 Bordeaux, France

Many cellular structures are too small to be resolved by conventional light microscopy. Two-photon microscopy is currently the method of choice for imaging in thick living tissue preparations (Denk 1990). However, the spatial resolution of a two-photon microscope, which is limited to ~350 nm by the diffraction of light, is not sufficient for resolving many important details of neural morphology (Ding 2009). To overcome this limitation, we have developed a two-photon excitation and pulsed stimulated emission depletion microscope, which provides unprecedented spatial resolution and excellent experimental access in acute brain slices (Hell 1994). The new microscope improves the spatial resolution of two-photon microscopy by a factor 4 - 6, and it is compatible with time-lapse and two-color live superresolution imaging. We demonstrate the potential of this nanoscopy approach for brain slice physiology by imaging the morphology of dendritic spines and microglial cells deep inside brain.

P6. Mehdi BHOURI

Bristol, United Kingdom
mehdi.bhouri@bristol.ac.uk

NMDA Receptor-mediated metaplasticity occurs selectively at schaffer collateral-CA1 synapses

Mehdi Bhouri[1]*, Paul A Farrow[2]*, Aneeta Motee, Xu Yan[3], Stephen M Fitzjohn[4], Zafar I Bashir[1]
1. MRC Centre for Synaptic Plasticity-school of Physiology and Pharmacology-University of Bristol-UK,
2. Synaptic Signaling and Neurodegeneration-German Cancer Research Centre - Heidelberg-Germany,
3. Neuroscience Center-University of Helsinki-FINLAND
4. Lilly UK-Surrey-UK

The hippocampus is a key structure of the brain for learning and memory processes. We know that two inputs to the CA1 region the Schaffer collaterals (SC) and the temporoammonic (TA) pathways have different role in these processes Indeed the SC-CA1 input is necessary for spatial memory encoding and short term spatial memory, whereas the TA-CA1 input is critical for long-term spatial memory consolidation and retrieval. N-methyl-D-aspartate receptors (NMDAR) are crucial for induction of synaptic plasticity and learning and memory processes, and are themselves subject to plasticity NMDAR-mediated plasticity can therefore change the threshold for induction of future synaptic plasticity and affect learning and memory. However, little is known about the effect of NMDAR-mediated plasticity on subsequent long-term potentiation (LTP) and long-term depression (LTD). Using a combination of whole-cell recordings and field recordings in the CA1 area of the

hippocampus, we have shown that the SC-CA1 input is subject to metaplasticity whereas the TA-CA1 input is not. Indeed a 5Hz 20s protocol induces LTD of NMDA receptor-mediated transmission in the SC-CA1 input but not in the TA-CA1 input. This LTD is mGlu receptor-dependent as it is blocked by application of LY311495, a group I mGluR antagonist. We have also shown that this LTD of NMDAR-mediated transmission prevents induction of LTP of AMPA transmission in the SC-CA1 input but not in the TA-CA1 input. However, LTD of AMPA transmission in both inputs is not affected by the LTD of NMDAR-mediated transmission. Therefore, these results show that the SC-CA1 input can undergo metaplasticity whereas the TA-CA1 input does not. These differences could explain the distinct roles of the two inputs in memory.

P7. Pierre BILLUART
Paris, France
pierre.billuart@inserm.fr

Cognitive Disorder Genes and Neuronal Morphology Characterization

J. Rucci[1], R. Montjean[1], M. Ramos[1], J. Renaud[1], F. Laumonnier[2], H. Stunnenberg[3], J. Chelly[1], O. Dorseuil[1] and P. Billuart[1]
1. Institut Cochin, INSERM U.1016, CNRS UMR.8104, Université Paris Descartes, Paris, France
2. INSERM U930, Faculté de Médecine, Tours, France
3. Radboud University, Nijmegen Centre for Molecular Life Science, Nijmegen, Netherlands

Recent discoveries on the genetic causes of intellectual disability (ID) or autism spectrum disorders (ASD) let appear that numerous ID/ASD genes encode i/ proteins directly observed at synapses or ii/ proteins located in the nucleus. During brain development, memory acquisition and learning, hippocampal neurons exhibit formation of highly complex and dynamic connectivity with up to thousands synapses per neuron. Conversely, these contacts appear altered in some ID or ASD situations of human pathology and in several mouse models of ID/ASD, suggesting a close association between ID/ASD and "synaptopathy". Pathology of synapses may therefore be considered as a hallmark of cognitive defects. To investigate this hypothesis, we study new ID genes discovered through the "Gencodys" network by systematic characterization of their ability to trigger "synaptopathy in a culture dish". Primary culture of mouse hippocampal neurons are differentiated in vitro and morphologically analyzed after down-regulating expression of each ID gene by RNA- interference technology. Potential defects at early and late stages of differentiation are evaluated by measuring neuritogenesis (dendritogenesis, axonogenesis) as well as synaptogenesis using fluorescent microscopy approaches. In parallel, correlations are searched for each ID gene between their phenotypes and its expression pattern during in vitro and in vivo neuronal differentiation. This approach will allow us to propose new clustering of ID/ASD genes integrating their morphological phenotype, their expression pattern during in vitro differentiation and their molecular function, with the aim of proposing new knowledge-based therapeutics.

P8. Andreas BJÖREFELDT
Gothenburg, Sweden
andreas.bjorefeldt@physiol.gu.se

The cerebrospinal fluid promotes excitatory neuronal activity

Björefeldt A, Andreasson U, Daborg J, Riebe I, Wasling P, Zetterberg H, Hanse E
Institute of Neuroscience and Physiology, University of Gothenburg, Sweden

Cerebrospinal fluid (CSF) bathes neurons in the brain and spinal cord and communicates freely with the interstitial fluid of the parenchyma. The CSF is known to contain numerous low molecular weight neuromodulators but how they collectively affect neuronal activity in the brain is unknown. We show here, by using a matched artificial CSF (aCSF) as control, that human CSF (hCSF) markedly increases both evoked and spontaneous excitatory activity of pyramidal cells in the in vitro hippocampal brain slice. While the increase in evoked transmission is associated with an increased release probability at CA3-CA1 presynaptic terminals, spontaneous neuronal activity is enhanced due to effects on intrinsic membrane properties. These include a lowered threshold for action potential firing (by about 5 mV), a depolarization of the resting membrane potential (about 3 mV) and a 20% decrease in input resistance of CA1 pyramidal cells. Spontaneous inhibitory GABAergic transmission is unaltered, indicating a differential effect of hCSF on excitatory and inhibitory

transmission. Our findings highlight a previously unrecognized activity-promoting role of CSF and suggest that recordings from neurons in in vitro brain slices can be made more in vivo-like by using physiological CSF.

P9. Paolo BOTTA
Basel, Switzerland
paolo.botta@fmi.ch

Tonic inhibition in central amygdala controls anxiety

Paolo Botta[1], Lynda Demmou[1], Chun Xu[1], Yu Kasugai[2], Michael Poe[3], James M. Cook[3], Uwe Rudolph4, Francesco Ferraguti[2], and Andreas Lüthi[1]
1. Friedrich Miescher Institute for Biomedical Research, Basel, Switzerland
2. Department of Pharmacology, Innsbruck Medical University, Innsbruck, Austria
3. Department of Chemistry, University of Wisconsin, Milwaukee, WI, USA
4. Laboratory of Genetic Neuropharmacology, McLean

The central nucleus of the amygdala (CEA) mediates the behavioral expression of conditioned fear responses. CEA output neurons, which are predominantly located in the medial subdivision (CEm), project to downstream targets in the brain stem and in the hypothalamus where they orchestrate conditioned autonomic and motor responses. CEm output neurons are under inhibitory control from GABAergic neurons located in the lateral subdivision (CEl). We recently found that fear conditioning is associated with changes in tonic activity of CEl and CEm neurons that correlate with fear generalization. The mechanisms underlying such changes in tonic activity are, however, unknown. Using whole-cell recordings from identified CEA neurons, we found that the activity of CEl and CEm neurons is controlled by a tonic current mediated by extrasynaptic GABAA receptors. Further, fear conditioning resulted in cell type-specific changes in tonic GABAA currents. Our findings suggest that learning-induced changes in tonic GABAergic inhibition is an important mechanism regulating fear and anxiety behavior.

P10. Paola CACCIN
Padova, Italy
paola.caccin@unipd.it

Effect of synaptic polybasic peptides on neurotransmission

Paola Caccin, Michele Scorzeto, Oriano Marin, Cesare Montecucco
Department of Biomedical Sciences, University of Padova, Italy

Some proteins of the complex neuroexocytosis machinery contain a small domain enriched in basic amino acids (lysines and arginines) located next to the transmembrane region and called juxtamembrane domain (JMD). For Synaptobrevin (VAMP), Syntaxin and Synaptotagmin, the JMD is very conserved among species, suggesting an important role in neuroexocytosis .In the present work, peptides mimicking the sequence of JMD of these three proteins, were synthetized with a hydrophobic residue (hexyle) bound to the native sequence. For comparative analysis, scrambled forms were also synthetized.The peptides were tested on mouse neuromuscular junction (twitch measurement using ex-vivo hemidiaphragm /phrenic nerve preparation), and by electrophysiological techniques, measuring the evoked potential in the muscular fibers, both in mouse and in Drosophila melanogaster.All peptides (concentration: 50-200 µM) were able to block the NMJ in a transient way, with a strong reduction of the evoked potential and a complete disappearance of spontaneous release; on the contrary, the scrambled forms had only a mild effect at the higher concentration.The second part of the work was then focused on synaptotagmin JMD peptide. It is well known that synaptotagmin is the calcium sensor for neurotransmitter release but the mechanism, by which synaptotagmin participates to the synaptic vesicles fusion, is not completely understood. Our hypothesis is that the positive residues in the JMD could bind phosphatidylserine in the synaptic vesicle, but also phosphatidylinositol 4,5-bisphosphate (PIP2) in the inner leaflet of the presynaptic membrane during the neuroexocytosis process, so contributing to an efficient fusion of the two membranes. To asses this point, in primary culture of motor neurons we used FM1-43 dye to monitor the synaptic vesicle cycle. Neurons were loaded and the unloading phase was monitored by fluorescent microscopy. In presence of different peptide concentrations, there was a significant reduction of SVs fusion rate.It was used also a fluorescent analogous of the synaptotagmin JMD peptide on the hemidiaphragm/phrenic nerve preparation, showing that the chemical modification does not abolished the peptide effect. Preliminary data showed a significant accumulation of the fluorescent-peptide in

correspondence to the NMJ and the afferent nerve.Taken together, these data suggest that polybasic peptides could interfere with neurotransmission and highlight the importance of the juxtamembrane domain of synaptotagmin that could have a specific role in the synaptic vesicles fusion process.

P11. Ana Luísa CARVALHO
Coimbra, Portugal
alc@cnc.uc.pt

Modulation of hippocampal glutamatergic synapses by ghrelin

Luís F Ribeiro[12], Tatiana Catarino[1], Sandra D Santos[1], J Fiona Van Leeuwen[12], José A Esteban[3], Ana Luísa Carvalho[12]

1. CNC-Center For Neuroscience and Cell Biology, University of Coimbra, Portugal

2. Department of Life Sciences, University Of Coimbra, Portugal

3. Centro De Biología Molecular Severo Ochoa, Consejo Superior De Investigaciones Científicas CSIC Universidad Autónoma de Madrid Madrid, Spain

Ghrelin is a peptide mainly produced by the stomach and released into circulation, affecting energy balance and growth hormone release. These effects are guided largely by the expression of the ghrelin receptor (GHS-R1a) in the hypothalamus and pituitary. However, GHS-R1a is expressed in other brain regions, including the hippocampus, where its activation enhances memory retention. We explored the molecular mechanism underlying the action of ghrelin on hippocampal-dependent memory. Our data show that GHS-R1a is localized in the vicinity of hippocampal excitatory synapses, and that its activation increases delivery of AMPA receptors (AMPARs) to synapses, producing functional modifications at excitatory synapses. Moreover, GHS-R1a activation enhances two different paradigms of long-term potentiation in the hippocampus, activates the phosphatidylinositol3-kinase and increases GluA1 AMPAR subunit and stargazin phosphorylation. We propose that GHS-R1a activation in the hippocampus enhances excitatory synaptic transmission and synaptic plasticity by regulating AMPARs trafficking. Our study provides insights into mechanisms that may mediate the cognition enhancing effect of ghrelin, and suggests a possible link between the regulation of energy metabolism and learning.

P12. Ronan CHEREAU
Bordeaux, France
ronan.chereau@u-bordeaux2.fr

STED imaging of axon morphology and plasticity

Ronan Chéreau[1,2], and U Valentin Nägerl[1,2]

1. Interdisciplinary Institute For Neuroscience, UMR 5297 CNRS Bordeaux

2. Université de Bordeaux, Bordeaux, France

Axons are the output station of neurons, rapidly conducting action potentials (AP) from the soma to thousands of downstream synapses. While it was thought for a long time that axons relay APs to presynaptic terminals in a digital and faithful manner, recent studies indicate that they in fact modulate APs in a highly complex and dynamic way, which may powerfully influence synaptic transmission, and hence neural circuit performance. Theoretical studies have long indicated that axon morphology may be a major determinant of how APs propagate in the axonal arbor. However, because unmyelinated axons in the brain are so poorly resolved by conventional light microscopy, it has been difficult to study axon morphology in living brain tissue. Hence, preciously little is known about how dynamic and plastic axons are under physiological conditions.To overcome this limitation, we have used STED microscopy and electrophysiological techniques in order 1) to characterize the dynamic morphology of CA3 axons (Schaffer collaterals) at the nanoscale under baseline conditions in organotypic hippocampal slice cultures and 2) to investigate the effects of neuronal activity on axon morphology.

P13. Amber CLAYTON

Oxford, United Kingdom
amber@strubi.ox.ac.uk

Structural and biophysical analysis of proteins interacting with the extracellular region of AMPA receptor subunits

Amber Clayton
Division of Structural Biology, University of Oxford

AMPA receptors (AMPARs) are tetrameric ionotropic glutamate receptors (iGluRs) containing combinations of four subunits termed GluA1-4. They are responsible for the majority of excitatory neurotransmission in the vertebrate central nervous system. The view of what exactly constitutes a physiological "AMPAR" is rapidly evolving, as the tetrameric core forming the actual ion channel seems merely to represent the central component of much larger supra-molecular assemblies. Multiple protein partners are thought to surround and modulate this core, but the stoichiometry, structural arrangement and functional consequences of such interactions are yet to be defined. We focused on four such molecules, putative interactors of the ~700 amino-acids AMPAR extracellular region: the neuronal pentraxins, CKAMP44, TARP2 (Stargazin) and Brorin. Biophysical analysis including surface plasmon resonance (SPR) and multi-angle light scattering (MALS) allow us to map their interaction sites. We present insights obtained from the high resolution crystal structures of the neuronal pentraxins as well as their oligomeric arrangements observed by single particle negative staining electron microscopy. These inspired a hypothesis whereby neuronal pentraxins, possibly secreted in an activity dependent manner, have the ability to control the surface trafficking and half-life of AMPARs. We validated this model in cellular assays and propose a general paradigm of iGluR regulation through receptor clustering.

P14. Claude COLOMER

Rotterdam, Netherlands
c.colomer@erasmusmc.nl

Action potential regulation of Arc expression

C Colomer, HR Geis, N Tanke, I Marcelo, JG G Borst, SA Kushner, A R
Erasmus MC Rotterdam Netherlands

The immediate-early gene Arc plays a key role in neuronal plasticity mechanisms underlying learning and memory. However, the exact relationship between neuronal activity and Arc expression has remained elusive. Here we used juxtacellular nanostimulation (Houweling & Brecht, 2008) to establish the relationship between action potential (AP) firing and Arc expression in single cortical pyramidal neurons in vivo. We systematically varied the number and frequency of APs in single layer 2 pyramidal cells in barrel somatosensory cortex of Arc-dVenus transgenic mice and subsequently monitored fluorescence of the Arc reporter using confocal microscopy. Fluorescence was observed after firing as few as 25-200 APs depending on the rate of AP firing. For a given firing rate expression increased monotonically with increasing AP numbers and approached asymptotic levels after 500 APs. The relationship between neuronal activity and Arc expression displayed a strong AP frequency dependence such that spike trains at lower firing rates required larger numbers of APs to induce Arc expression and resulted in lower asymptotic levels. Control experiments showed that nanostimulation-induced Arc expression required action potentials and was not dependent on excitatory synaptic inputs. Additional experiments in which brief nanostimulation trials were applied at regular time intervals suggest that AP activity can be faithfully integrated over periods of time lasting up to tens of minutes. Together, we demonstrate that Arc expression is tightly regulated by neuronal activity over a wide range of time scales, from seconds to minutes. Moreover, these results are the first to establish a quantitative transfer function between in vivo neuronal activity and gene expression, at the single cell level.

P15. Sarah COUSINS

London, United Kingdom
s.cousins@ucl.ac.uk

NETO-1 associates with the NMDA receptor/APP protein complex

Sarah L. Cousins, Neal Innocent, and F. Anne Stephenson
UCL School of Pharmacy, London, WC1N 1AX, UK

Neuropilin tolloid-like 1 (Neto1), is a CUB domain-containing transmembrane protein that was recently identified as a novel component of the NMDA receptor complex where it was shown to be critical for maintaining the abundance of GluN2A-containing NMDARs in the postsynaptic density [1]. We have previously shown that assembled NMDA receptors form a complex with amyloid precursor protein (APP) [2] thus it was of interest to determine if Neto1 was a constituent of the APP695/GluN1/GluN2A and APP695/GluN1/GluN2B) trafficking complexes. Neto1HA was shown to co-immunoprecipitate with assembled NMDA receptors via GluN2A or GluN2B subunits; Neto1HA did not co-immunoprecipitate APP695FLAG. Co-immunoprecipitations from mammalian cells co-transfected with APP695FLAG, Neto1HA and GluN1/GluN2A or GluN1/GluN2B revealed that all four proteins co-exist within one macromolecular complex. Immunoprecipitations from native brain tissue similarly revealed the existence of a GluN1/GluN2A or GluN2B/APP/Neto1 complex. Neto1HA caused a reduction in the surface expression of both NMDA receptor subtypes, but had no effect on APP695FLAG- or PSD-95ac-Myc enhanced surface receptor expression. The Neto1 binding domain of GluN2A was mapped using GluN1/GluN2A chimeras and GluN2A truncation constructs. The extracellular GluN2A domain does not contribute to association with Neto1HA but deletion of the intracellular tail resulted in a loss of Neto-1HA co-immunoprecipitation which was paralleled by a loss of association between GluN2A and SAP102. Thus, Neto1 is concluded to be a component of APP/NMDA receptor trafficking complexes [3].

[1] Ng, D., Pitcher, G.M., Szilard, R.K., Sertie, A., Kanisek, M., Clapcote, S.J., Lipina, T., Kalia, L.V., Joo, D., McKerlie, C., Cortez, M., Roder, J.C., Salter, M.W. and McInnes, R.R. (2009) PLoS Biology 7, 0278-0300.
[2] Cousins, S.L., Hoey, S.E., Stephenson, F. A. and Perkinton, M.S. (2009) J. Neurochem. 111,1501-1513.
[3] Cousins, S.L., Innocent, N. and Stephenson, F.A. (2013) J. Neurochem. In the press.

P16. Patrizia D'ADAMO

Milan, Italy
p.dadamo@hsr.it

The in vitro and in vivo characterization of RAB39B: an intellectual disability and autism spectrum disorder protein controlling the ER-exit of GluA2 AMPAR and cognitive functions

Giannandrea M[1,2]*, Mignogna ML[1,3]*, Moré L[1], Bianchi V[1,3], Fanelli F[4], Mapelli L[5], Bassani S[5], Passafaro M[5], Gatti McArthur S[2], Huganir RL[6], Esteban JA[7], D'Adamo P[1,3]
1. Dulbecco Telethon Institute (DTI) at San Raffaele Scientific Institute, Milan, Italy
2. F Hoffmann-La Roche AG, pRED Pharma Research & Early Development DTA CNS, Basel, Switzerland
3. University Vita-Salute San Raffaele, Milan, Italy
4. DTI at University of Modena and Reggio Emilia, Modena, Italy

Intellectual Disability (ID) is a common developmental brain disorder characterized by an IQ lower than 70. About 2% of the population is affected by ID and the disease can be comorbid with autistic spectrum disorders (ASD) with a very severe social and economic impact. The goal of our group is to identify new genes responsible for ID to understand which are the molecular pathways involved in the learning and memory formation. We focus genomic studies on specific intracellular pathways linked to ID and particular attention was done to genetic deficits affecting RAB GTPases and RAB-interacting proteins. We discover two genes, GDI1 and RAB39B, functionally related, mutated in ID families. Indeed, GDI1 encodes for αGDI that is one of the proteins controlling the cycling of RAB GTPase involved in intracellular vesicular traffic, pointing to an important role of αGDI and RAB39B in cognitive function. After the discovery of loss of function mutations in the RAB39B gene in families affected by ID and ASD, and a first functional characterization involving RAB39B in neuronal development and synapse formation and maintenance, we asked for the specific role of RAB39B in intracellular trafficking. We further characterize the functional role of RAB39B in neuronal cells, demonstrating that RAB39B is involved in the secretory endoplasmic reticulum (ER)-to-Golgi pathway of the GluA2-containing AMPAR through its specific interaction with PICK1. Rab39b silencing leads to alteration in the GluA2 secretory pathway and, as a final step, to a reduction in the steady state amount of GluA2 AMPAR subunit at the post-synaptic

sites, leading to the formation of those Ca2+-permeable AMPARs often associated with immature synapsis. These findings identify RAB39B as a key molecule in mediating the translocation of GluA2-containing AMPARs from ER-to-Golgi. Ultimately, the study provides an explanation for the involvement of RAB39B mutations in the ethiology of ID/ASD. Finally, to understand the RAB39B function in synaptic plasticity and cognition, we generated a Rab39b knock down (KD) mouse. Rab39b KD mice are viable and fertile and did not present macroscopic morphological or neuropathological alteration. An extensive behavioural characterization points to a deficit in the first learning phase of water maze and radial maze tests. Rab39b KD mice show a marked reduction in social interaction in the 3-chambers sociability test, a validated test for ASD phenotype. All togheter these first results underly the pre-clinical validity of this animal model and it provides a clear advantage to discover new therapeutic strategies targeting a deficit in AMPA receptor function.

P17. Jary DELGADO

Bordeaux, France
jary.delgado@u-bordeaux.fr

Pin1-mediated phosphorylation-dependent prolyl isomerization regulates excitatory synapses via a PSD-95 N-terminus conformational change

Jary Y Delgado, and Daniel Choquet
Interdisciplinary Institute For NeuroScience, UMR 5297, Université de Bordeaux

Phosphorylation dependent peptidyl-propyl cis/trans isomerization by the Peptidyl-prolyl cis-trans isomerase NIMA-interacting 1 (Pin1) plays a key role in cell cycle progression, the pathogenesis of cancer, long-term potentiation and age-related neurodegeneration. While most of our knowledge is restricted to protein targets outside brain tissue, the specific ways by which Pin1 regulates excitatory synapse maintenance, synaptic transmission and synaptic plasticity are yet unknown. Here we identify the Postsynaptic Density Protein 95 (PSD-95) protein as a novel Pin1 binding partner. Pin1 preferentially binds to phosphorylated threonine 19 (T19) and serine 25 (S25), two sites known to regulate PSD-95 multimerization, ubiquitination and endocytosis of AMPA receptors, via its N-terminus WW domain. Furthermore, PSD-95 phosphorylation increases cis N-terminus phosphorylated PSD-95 of which Pin1 isomerises from cis to trans the prolyl bonds via its C-terminal isomerase domain. Binding of Pin1 to PSD-95, both in vitro and in vivo, prevents PP2A binding and dephosphorylation of T19. Given that T19 and S25 phosphorylation regulates PSD-95 multimerization we tested whether Pin1 binding interferes with this process. As expected, downregulation of Pin1 increases the amount of multimeric PSD-95 as measured from CO-IP assays but paradoxically reduced the frequency of PSD-95 containing synapses along dendritic spines and increase the amount of overexpress extrasynaptic PSD-95. This requires the constitutive activity of the ubiquitin-proteosome pathway which is also responsible for an observed increase in the mobile pool of PSD-95::EGFP, as measured by FRAP. Finally, we tested the effects of Pin1 on AMPAR mediated excitatory synaptic transmission and found that Pin1 inhibition reduces the frequency but not the amplitude of mEPSCs while overexpression of the WW domain of Pin1 or an isomerase deficient mutant decrease the amplitude of the mEPSCs but increase their frequency. Our results are the first to demonstrate the precise series of signaling events following T19 and S25 phosphorylation which in turn regulate PSD-95 life-time at excitatory synapses.

P18. Julien DUPUIS

Bordeaux, France
julien.dupuis@u-bordeaux2.fr

Single molecule crosstalk between surface NMDA and dopamine D1 receptors tunes plasticity at hippocampal excitatory synapses

Laurent Ladépêche[1,2], Julien Pierre Dupuis[1,2], Delphine Bouchet[1,2], Evelyne Doudnikoff[3], Luting Yang[1,2], Yohan Campagne[1,2], Hélène Gréa[1,2], Erwan Bézard[3], Eric Hosy[1,2] and Laurent Groc[1,2].
1. Univ. Bordeaux, Interdisciplinary Institute For Neuroscience, UMR 5297, F-33000 Bordeaux, France
2. CNRS, Interdisciplinary Institute For Neuroscience, UMR 5297, F-33000 Bordeaux, France
3. Univ. Bordeaux, Institute of Neurodegenerative Diseases, UMR 5293, F-33000 Bordeaux, France

Dopamine is a powerful modulator of glutamatergic neurotransmission and N-methyl-D-aspartate receptor (NMDAR)-dependent synaptic plasticity. Although several intracellular cascades participating in this functional

dialogue have been identified over the last decades, the molecular crosstalk between surface dopamine and glutamate NMDA receptors-associated signalling still remains poorly understood. Using a combination of single nanoparticle detection imaging and electrophysiology in live hippocampal neurons, we here unravel that dopamine D1 receptors (D1R) and NMDAR form dynamic surface clusters in the surrounding of glutamate synapses. Strikingly, D1R activation or D1R/NMDAR interaction cleavage decreases the size of these clusters and increases NMDAR synaptic content through a fast lateral redistribution of the receptors, thereby enhancing long-term synaptic potentiation. Together, these data reveal the unexpected presence of dynamic D1R/NMDAR perisynaptic reservoirs favoring a rapid and bidirectional surface crosstalk between receptors, and set the plasma membrane as the primary stage of the dopamine-glutamate interplay.

P19. Sabine FIEVRE
Bordeaux, France
sabinekfievre@hotmail.fr

Molecular determinants for targeting of glutamate receptors at hippocampal mossy fiber synapses

Fièvre S, Carta M, Coussen F, Mulle C
Univ. Bordeaux, Institut interdisciplinaire de neurosciences, UMR 5297, F-33000 Bordeaux, France
CNRS, Institut interdisciplinaire de neurosciences, UMR 5297, F-33000 Bordeaux, France

Trafficking and stabilization of ionotropic glutamatergic receptors (AMPA, NMDA and kainate receptors (KARs)) at synaptic sites involve interactions with different partners including scaffolding proteins and auxiliary subunits. These interactions occur through different receptor domains, including the C-terminal domain (CTD). In heterogous culture systems or in cultured dissociated neurons, surface trafficking of kainate receptors involves the 39 last amino acids of the CTD. KARs are specifically expressed in the stratum lucidum where mossy fibers contact CA3 pyramidal cells (Mf-CA3 synapses), whereas they are absent from other glutamatergic synaptic inputs to the same neuron. The mechanisms for such a constrained subcellular segregation is not known. At Mf-CA3 synapses, KARs comprise the GluK2, GluK4 and GluK5 subunits. Genetic ablation of GluK2 induces the loss of synaptic KAR-mediated currents. To investigate the role of the CTD of GluK2 in the synaptic trafficking and stabilization of KARs at mossy fiber-CA3 synapse we used a molecular replacement strategy in organotypic hippocampal cultures combined with electrophysiology and focal glutamate uncaging. Reexpression of GluK2wt in CA3 pyramidal cells of GluK2-/- mice specifically restores KAR-mediated currents at Mf-CA3 synapses and not at other glutamatergic inputs. By reexpression of mutant and truncated GluK2 subunits, we have identified a region in the CTD of GluK2 necessary for the synaptic trafficking and stabilization of KARs at Mf-CA3 synapses. We are currently performing glutamate uncaging coupled to patch clamp recordings to determine a functional map of KARs along CA3 pyramidal cells dendrites. Our study provides evidence for the molecular mechanisms underlying the stringent segregation of glutamate receptor subtypes at specific glutamatergic synapses in the hippocampal circuit.

P20. Maria FIUZA
Pamplona, Spain
mfiuza@unav.es

GluN3A expression restricts spine maturation via inhibition of GIT1/Rac1 signaling

Maria Fiuza, Immaculada Gonzalez-Gonzalez, Isabel Perez-Otano
Cellular Neurobiology Laboratory, Centro de Investigación Médica Aplicada (CIMA)

NMDA-type glutamate receptors (NMDARs) guide the activity-dependent remodeling of excitatory synapses and associated dendritic spines during critical periods of postnatal brain development. Whereas mature NMDARs composed of GluN1 and GluN2 subunits mediate synapse plasticity and promote spine growth and stabilization, juvenile NMDARs containing GluN3A subunits are thought to inhibit these processes via yet unknown mechanisms. Here we report that juvenile NMDARs containing GluN3A bind GIT1, a postsynaptic scaffold that assembles actin regulatory complexes including the Rac1 guanine nucleotide exchange factor βPIX to promote Rac1 activation in spines. Binding to GluN3A limits the synaptic localization of GIT1 and its ability to complex with βPIX, leading to decreased Rac1 activation and reduced spine density and size in primary cultured neurons. Conversely, knocking out GluN3A favors the formation of GIT1/βPIX complexes and increases the activation of Rac1 and its main effector p21-activated kinase (PAK). We further show that binding of GluN3A to

GIT1 is regulated by synaptic activity, a response that might restrict the negative regulatory effects of GluN3A on actin signaling to inactive synapses. Our results identify inhibition of Rac1/PAK actin signaling pathways as an activity-dependent mechanism mediating the inhibitory effects of GluN3A on spine morphogenesis.

P21. Yoshiyuki FUKUDA
Bayern, Germany
fukuda@biochem.mpg.de

Exploring synaptic architecture by cryo-electron tomography of FIB-milled neuronal cells

Yoshiyuki Fukuda, Miroslava Schaffer, Alexander Rigort, Wolfgang Baumeister, Vladan Lučić
Department of Molecular Structural Biology, Max Planck Institute of Biochemistry

To understand synaptic function, unveiling molecular architecture of synapse is one of the important issues. Transmission electron microscopy observation of plastic embedded specimens stained with heavy metals has fundamentally contributed to our knowledge about basic structural features of neuronal synapses. However, structural investigations of synapse at the close-to-physiological state are required for a further understanding of synaptic architecture and function. Cryo-electron tomography (CET) of vitrified, frozen-hydrated cells is a rapidly developing method for three-dimensional visualization of cellular structures preserved in their natural, cellular environment. However, applications of this method are typically restricted to peripheral cellular regions up to approximately 500 nm in thickness. Therefore, it is difficult for CET to observe synapse directly. Focused ion beam (FIB) milling is a method that allows thinning of vitrified specimens and thus enables CET observations of the entire cellular interior. In this study, we show high resolution images of synapses preserved at the close-to-physiological state, by combining CET and FIB. Primary hippocampal neuronal cells were cultured for 17-18 DIV on EM specimen grids and plunge-frozen by a rapid immersion into liquid ethane. Tilt-series images of vitrified synapses were acquired at 300 kV and subsequently reconstructed. These reconstructed tomograms clearly showed fine structures such as tethers linking synaptic vesicles to the active zone, synaptic adhesion complexes in the synaptic cleft, the postsynaptic density and actin filaments. Furthermore, reconstructed tomograms were segmented to allow quantitative analysis of these structures. We expect that the combined application of CET and FIB to vitrified, frozen hydrated neuronal cells will contribute to investigations of synaptic architecture in intact cells at the close-to-physiological state for further understanding of synaptic function.

P22. Frédéric GAMBINO
Geneva, Switzerland
frederic.gambino@unige.ch

Nonlinear paralemniscal thalamic inputs mediate whisker-evoked heterosynaptic plasticity in the somatosensory cortex

Frédéric Gambino, Vasileios Kechagias, Stéphane Pagès, Anthony Holtmaat
Department of Basic Neurosciences, Medical School of Geneva, Geneva Neuroscience Center

Long-term potentiation (LTP) of synaptic connections within layer (L) 2/3 of the barrel cortex is thought to underlie sensory experience-dependent cortical map plasticity. One possible mechanism for LTP involves the temporal coincidence of synaptic inputs and somatic action potentials (APs) that back-propagate into dendrites, termed spike-timing dependent (STD) LTP. Indeed, we have previously shown that experience-mediated disinhibition facilitates STD-LTP in L2/3 pyramidal neurons in the barrel cortex. However, spontaneous and sensory-evoked spiking is strikingly sparse in those L2/3 pyramidal neurons. This casts doubt as to whether repetitive and natural whisking would ever produce enough somatic APs to induce LTP. Here we show using in vivo whole-cell recording, calcium imaging, and optogenetics in the anesthetized mouse, that repetitive whisker deflections can induce LTP of whisker-mediated synaptic inputs without generating somatic APs. This potentiation was specific to the stimulated whisker, and it required nonlinear NMDARs-dependent plateau depolarization driven by inputs that are relayed in the posterior medial (POm) higher order thalamic nucleus. These inputs are part of the paralemniscal pathway and project in a non-specific manner to the distal dendrites of L2/3 neurons. Together, our data suggest that the activation of paralemniscal somatosensory pathways facilitates the strengthening of spatially segregated synaptic inputs on barrel cortex pyramidal cells.

P23. Maurice GARRET
Bordeaux, France
maurice.garret@u-bordeaux2.fr

Alteration of GABAergic neurotransmission within striatum and globus pallidus in R6/1 Huntington disease model

Zhuowei Du, Marine Chazalon, Jerome Baufreton, Yoon Cho, Maurice Garret
Univ. Bordeaux, INCIA, CNRS-UMR 5287, F-33000 Bordeaux, France, Univ. de Bordeaux, Institut des Maladies Neurodégénératives, CNRS-UMR 5293, F-33000 Bordeaux

GABA is the major inhibitory neurotransmitter in the brain and it is well established that alterations of GABAergic neurotransmission occur in many neurological disorders such as Huntington's disease. This disease is caused by a mutation in the huntingtin gene leading to dysfunction in the Caudate Putamen (CPu). The CPu and Globus Pallidus (GP) are part of the basal ganglia circuit that plays an important role in the control of movement and contain almost exclusively GABAergic projecting neurons. Therefore, movement disorders in Huntington's disease might be linked to alterations in GABAergic neurotransmission in CPu and GP. In this study, the R6/1 mouse model expressing the mutated human HD gene is used. The HD symptoms, such as clasping, appear at about 5 months in this model. There is also significant body weight loss and CPu atrophy at 6 months. To assess whether the GABAergic neurotransmission is affected in R6/1 mice, we first analyzed the expression of several proteins known to be present in GABAergic synapses. These proteins include GABAAR subunits (α1, β2, β3), scaffolding proteins (NL2, Gephyrin), and presynaptic proteins (VGAT, GAD65 and 67). Expression levels were measured by both Western blot analysis and immunocytochemical labeling. WT and R6/1 littermates were used at 2 ages: pre-symptomatic (2 months) and symptomatic (6 months). In symptomatic mice, we found that the expression level of several GABAAR subunits was increased in the CPu, thus explaining functional analysis data previously published. On the contrary, the expression level of several markers, including GABAAR subunits, was decreased in the GP. These data suggest a profound modification of the CPu-GP pathway in 6 month-old symptomatic R6/1 mice. Furthermore and surprisingly, the same pattern of expression changes was also found in both CPu and GP at a early pre-symptomatic age. Electrophysiological analysis, using patch clamp recording showed that the decrease in expression level in the GP in 2 month old mice is associated with a decrease in miniature and spontaneous inhibitory post-synaptic currents. In order to analyze in depth alterations of GABAergic synapses, we are developing new tools to evaluate synaptic connections within the GP of R6/1 mice and wild type littermates. These tools include uniform sampling of brain tissue, immunohistochemistry, mosaic scanning with confocal microscope, Huygens deconvolution and Imaris analysis. This study uncovered an unexpected alteration of the GP in Huntington disease.

P24. Jimmy GEORGE
Bordeaux, France
mails2jimmy@yahoo.in

Microglial Adenosine 2a receptor modulates MF-CA3 synaptic transmission via the release of extracellular ATP

Jimmy George, Christophe Mulle, Rodrigo Cunha, Thierry Amédée
University de Bordeaux, Interdisciplinary Institute for Neuroscience, Bordeaux, France, Centre for Neuroscience and Cell Biology, Univeristy of Coimbra, Coimbra, Portugal.

Microglial cells play an important role in the immune reactions associated with the central nervous system. They are known to get activated due to ATP released by astrocytes and also by damaged neurons. Thus ATP acts as a chemo attractant to microglial cells in the site of injury. We have previously found that activation of microglia with inflammatory stimuli like LPS and Glutamate is associated with changes in the extracellular ATP levels, an effect modulated by microglial A2a receptor. In keeping with the above findings, the aim of the present work is to study how microglial cells can affect synaptic transmission and plasticity at the mossy fiber - CA3 synapse in hippocampal slices. To do so, microglial cell line (N9 cells) were first activated by LPS then added on top of acute hippocampal slices. Synaptic transmission were recorded from CA3 pyramidal cells in the whole-cell voltage-clamp mode using minimal intensity stimulation of mossy fibers. Preliminary results indicate a decrease in synaptic transmission when slices were co-incubated with LPS activated N9 cells. This decrease was prevented when A2a receptor antagonist, [SCH58261 (50nM)] was added prior to LPS on N9 cells. The

present results when combined with our previous work suggests that the ATP released from microglia on stimulation with LPS acts on purinergic receptors located on either pre or post synaptic locii resulting in the change in synaptic transmission. Further work will help us in better understanding the role of extracellular ATP, its metabolites and its respective receptors in the governance of synaptic transmission associated with inflammation in neurodegenerative diseases. (Supported by ENC).

P25. Adam GORLEWICZ

Bordeaux, France
adam.gorlewicz@gmail.com

The role of kainate receptors in acute models of epilepsy

Adam Gorlewicz, Stefano Zucca, Noelle Grosjean, Peggy Vincent, Christophe Mulle
Interdisciplinary Institute for Neuroscience, University of Bordeaux, and CNRS UMR 5297, F-33000 Bordeaux, France

Epilepsy is a chronic neurological disorder that is characterized by the occurrence of spontaneous seizures. A seizure appears when neurons of a certain area of the brain (epileptic foci) are activated in an untypically synchronous manner and spread abnormal activation pattern into connected brain regions. The presented study concentrates on the role of kainate receptor in propagation and the modulation of epileptogenic actions in mouse hippocampus in acute seizures caused by systemic injection of proconvulsants. Physiological properties of kainate receptors (KARs) have been widely studied at mossy fiber synapses on CA3 pyramidal cells in the hippocampus where presynaptic mossy fibers from dentate gyrus (DG) granule cells (GC) invade the lowermost apical dendrite of CA3 principal cells making complex synapses with a distinctive histological pattern known as stratum lucidum. In stratum lucidum KARs are composed of four subunits (GluK1, GluK2, GluK3, GluK5), which can form functional homomeric and heteromeric channels. They exert their function by acting at either pre- or postsynaptic sites, modulating the balance between excitation and inhibition, which suggests that KARs may selectively participate in the development of epileptiform dysfunction in hippocampal network. In the presented project we have analysed the susceptibility of different KAR subunits “knock out” strains of mice to seizures, that were induced by systemic administration of three different epileptogenic compounds (kainite, pilocarpine and metrazol), each with distinct mechanisms of action. Our current results demonstrate an important role of GluK2 subunit in controlling the proper excitability in CA3 region of hippocampus after pilocarpine injection. Moreover the preliminary data also suggests the importance of GluK2 for excitotoxic effect of pilocarpine administration.

P26. Laurie-Anne GOUTY-COLOMER

Rotterdam, Netherlands
l.colomer-gouty@erasmusmc.nl

Arc expression identifies the neuronal ensemble within the lateral amygdala recruited during fear conditioning

Gouty-Colomer L-A[1], Hosseini B[1], Colomer C[2], Marcelo I[1], Schreiber J[2], Slump D[1], Jaarsma D[2], Houweling A[2], Elgersma Y[2], Kushner SA[1]
Erasmus University Medical Center
1. Dept of Psychiatry,
2. Dept of Neuroscience, Rotterdam, The Netherlands

The biology of memory is among the most fundamental questions in modern neuroscience. Fear conditioning induces a profound memory with a robust behavioral phenotype, gated by changes in synaptic plasticity within the lateral amygdala. However, further progress in defining the precise molecular and cellular mechanism has been limited by the sparse encoding of fear memory, since only a minority of lateral amygdala neurons change their tone-evoked activity in vivo after auditory fear learning. Moreover, a similar proportion of neurons express the immediate-early gene Arc after fear conditioning, consistent with its well-known role in memory consolidation. However, the precise electrophysiological signature of the Arc-expressing ensemble has remained an important unanswered question. Therefore, we used Arc-dVenus reporter mice to perform confocal stereology and targeted whole-cell patch-clamp recordings of lateral amygdala neuronal ensembles specifically recruited during fear learning. Stereological quantification and fluorescence analysis of dVenus+

neurons show that a single tone-shock pairing is sufficient to induce the expression of Arc in the lateral amygdala. With increasing strength of fear conditioning, we observed the enhanced recruitment of dVenus+ neurons with a significant rightward-shift in the cumulative distribution of fluorescence intensity. Using fluorescence-targeted whole-cell recordings, we found that Arc+ neurons have increased excitability compared to their Arc- neighbors, even under naïve conditions, and independent of sensory stimulation or learning. In contrast, fear learning induced a robust, post-synaptically mediated potentiation of excitatory thalamic afferent synapses, selectively in the Arc+ cell population. Notably, these changes were highly specific to fear learning, as they were absent under naïve conditions or following unpaired stimulus presentations. Moreover, the increase in excitatory postsynaptic currents was localized post-synaptically, given an increased AMPA/NMDA ratio and the absence of any changes in the paired-pulse ratio. In conclusion, we show learning-induced synaptic plasticity in the lateral amygdala is selectively localized to Arc-expressing cells. Taken together, our results suggest that Arc expression provides a unique molecular marker for identifying neuronal ensembles functionally participating in memory encoding.

P27. Agata GOZDZ
Warsaw, Poland
agozdz@iimcb.gov.pl

GSK3a/b affect Arc protein expression in primary neuronal culture

Gozdz A., Cymerman I., Urbanska M., Jaworski J.
International Institute of Molecular and Cell Biology, Warsaw, Poland

GSK3 alpha/beta (Glycogen Synthase Kinases alpha/beta) are serine/threonine kinases ubiquitously expressed in the nervous system. GSK3's appear to be critical for LTD formation and overactivation of GSK3 inhibits LTP expression. Here we report that GSK3 regulate expression of Arc/Arg3.1 protein (Activity Regulated Cytoskeleton Associated Protein/Activity Regulated Gene 3.1), involved in diverse forms of synaptic plasticity, including LTP, LTD and homeostatic plasticity. We hypothesize that Arc could be one of the putative GSK3 effectors in neurons. The combination of low dose NMDA and GSK3 inhibitors up-regulated Arc expression at the protein but not at the mRNA level. Recombinant Arc protein is phosphorylated in vitro by GSK3 beta. Currently, we are characterizing the mechanism of GSK3- dependent Arc degradation. We also observed that the co-treatment of neurons with GSK3 inhibitors and NMDA induced alterations in the dendritic spine morphology. We are employing shRNA technology to determine if Arc contributes to the observed alterations in dendritic spines morphology and what is the role of GSK3- dependent Arc degradation in different forms of synaptic plasticity. Supported by 7FP EU grant 223276 “NeuroGSK3” and NCN 05397 grant from National Center for Science.

P28. Marilena GRIGUOLI
Bordeaux, France
marilena.griguoli@u-bordeaux2.fr

Cholinergic modulation of intact CA3 circuits in vivo

Griguoli M, Zucca S, and Mulle C
Interdisciplinary Institute for Neuroscience, University of Bordeaux, and CNRS UMR 5297, F-33000 Bordeaux, France

Hippocampus receives extensive cholinergic innervation from fibers mainly originating in the medial septal nucleus of the basal forebrain. Acetylcholine (ACh), released by cholinergic fibers, targets nicotinic and muscarinic receptors distributed in all hippocampal regions including CA3 neurons, which are particularly involved in new memory encoding. The relevance of the hippocampus and the CA3 region for memory processes and the well known importance of the cholinergic system for cognitive functions make it necessary to better understand how the activity of CA3 circuits is controlled by ACh in the intact network of a mammalian brain. To address this question we used an optogenetic approach, to selectively control the activity of cholinergic neurons, combined with in vivo recordings from CA3 pyramidal neurons. Double-floxed inverted open reading frame (DIO) viral vectors were used to target the expression of ChR2-EYFP to the cholinergic neurons of the medial septum in ChAT-Cre mice. To test the functionality of ChR2 we first performed whole cell recordings from EYFP positive cholinergic cells in acute brain slices. Brief pulses of blue light (0.5-1 ms; 470 nm) reliably induced action potentials at different frequencies of stimulation, indicating that this population of

cholinergic neurons can be optically controlled. To study how the activation of the cholinergic neurons modulate CA3 network activity in the anesthetized mouse, an optical fiber was acutely implanted in the medial septum to deliver optical stimulation (1-5 ms, 470nm). We then recorded multi unit activity and oscillatory network activity (theta and gamma bands) in basal conditions and after light-mediated cholinergic stimulation. Activation of the cholinergic system increased the firing frequency of CA3 neurons and was associated with a consistent increase in the power of theta oscillations. Further studies at the single cell level will allow us to better characterize the effect of cholinergic activation on intrinsic membrane properties and synaptic inputs. This study will gain new insights on the functional role of ACh in modulating CA3 circuits involved in the rapid encoding of new information.

P29. Matthias HABERL

Bordeaux, France
matthias.haberl@inserm.fr

Anterograde tracing using a novel envelope-switched ΔG rabies virus variant

Haberl M*, Viana Da Silva S*, Guest M Ginger M, Mulle C, Oberlaender M, Conzelmann KK, Frick A
Equal contributions
University of Bordeaux France, University of Zurich, Switzerland

An understanding of how the brain processes information requires knowledge of the architecture of its underlying neuronal circuits, as well as insights into the relationship between architecture and physiological function. A range of sophisticated tools is needed to acquire this knowledge, and recombinant rabies virus (RABV) is becoming an increasingly important part of this essential toolbox. The glycoprotein deleted (ΔG) RABV has permitted a range of approaches for the study of defined neuronal circuits. Here we report a novel RABV ΔG vector that infects cell bodies permitting anterograde tracing of fine-detailed neuronal structure. We will show how this technique can be applied in various brain regions in combination, and for sparse labeling combined with a variety of imaging/analysis tools to gain knowledge of neuronal morphology and connectivity. This vector can be readily combined with retrograde labeling or mono-trans-synaptic tracing, complementing the existing toolbox for dissecting brain anatomy and physiology.

P30. Etienne HERZOG

Bordeaux, France
etienne.herzog@u-bordeaux2.fr

Vesicular glutamate transporters as markers and players of synaptic vesicle mobility

Kätlin Silm[1], Léa Siksou[2], Fabien Nadrigny[3], Xiaomin Zhang[4], Serge Marty[2], and Etienne Herzog[1,4,5,6]
1. UPMC - INSERM U952
2. IBENS - INSERM U1024
3. MPIEM Dept of Neurogenetics
4. Univ Bordeaux, Interdisciplinary Institute For Neuroscience, UMR 5297, F-33000 Bordeaux, France
5. CNRS, Interdisciplinary Institute For Neuroscience, UMR 5297, F-33000 Bordeaux, France
6. MPIEM Dept of Molecular Neurobiology

The vesicular glutamate transporter VGLUT1 loads synaptic vesicles with the neurotransmitter glutamate and thereby determines glutamate release at many synapses in the mammalian brain. Due to its function and selective localization, VGLUT1 is one of the most specific markers for glutamatergic synaptic vesicles. We recently generated a fluorescent VGLUT1VENUS knock-in mouse to show that synaptic vesicles are dynamically shared among boutons in the cortex of mice in vivo. We further provided a detailed analysis of synaptic vesicle sharing in vitro, and show that network homeostasis leads to dynamic scaling of synaptic VGLUT1 levels. While the essential function of VGLUTs as glutamate transporter has been well established, the evidence for additional cell-biological functions is more controversial. Both VGLUT1 and -2 disruptions in mice were reported to result in a reduced number of SVs, a flattening of SVs, and the appearance of tubular structures in terminals. Our current analysis using high-pressure freezing immobilization and electron tomography confirm the steep reduction in the number of SVs previously observed in VGLUT1-/- presynaptic terminals, but we do not observe accumulation of endocytotic intermediates. SV proteins were found to be expressed at normal

levels in homogenates, synaptosomes and SV fractions of adult VGLUT1-/- mice, ruling out the possibility of a massively altered membrane trafficking. We thus assessed the mobility of SVs using Synaptobrevin2-EGFP and revealed an oversized super-pool of SVs in VGLUT1-/- neurons. Our results therefore support that beyond glutamate loading, VGLUT1 might stabilize synaptic vesicles at presynaptic terminals.

P31. Julie JEZEQUEL

Bordeaux, France
julie.jezequel@etud.u-bordeaux2.fr

Altered surface interplay between NMDA and dopamine receptors in a neuropsychiatric disorder

Julie Jézéquel[1,2], Lenka Mikasova[1,2], Delphine Bouchet[1,2], Véronique Rogemond[3], Jérôme Honnorat[3], Laurent Groc[1,2]

1. Univ. Bordeaux, Interdisciplinary Institute for Neuroscience, UMR 5297, F-33000 Bordeaux, France
2. CNRS, Interdisciplinary Institute for Neuroscience, UMR 5297, F-33000 Bordeaux, France
3. Lyon Neuroscience Research Center INSERM U1028/CNRS UMR 5292, F-69372 Lyon

Glutamatergic N-Methyl-D-Aspartate receptors (NMDAR) play a key role in many physiological processes. A disrupted balance between the NMDAR and dopamine receptor signaling has been involved in major neuropsychiatric disorders. The most described synaptic autoimmune encephalitis is associated with autoantibodies directed against extracellular domains of the NMDAR, leading patients to develop severe psychotic symptoms. Although NMDAR are the primary target of these antibodies, the cellular and molecular pathway(s) that conduct to their dysfunction remain to be fully understood. Here, we investigated the impact of such autoantibodies on the surface trafficking of NMDAR and dopamine receptors using a combination of high resolution nanoparticule imaging and immunocytochemistry. We report that anti-NMDAR antibodies from patients with encephalitis strongly disturb the surface content and trafficking of the receptor in cultured hippocampal neurons. Surprisingly, the anti-NMDAR antibodies also alter the surface dynamics of the dopaminergic D1 receptors, which is known to directly interact with the GluN1 subunit of the NMDAR. This suggests that an altered trafficking of the primary target of an autoantibody, i.e. against NMDAR, potentially triggers a “domino effect” on associated membrane receptors. Together, these data show that an altered surface trafficking of NMDAR, induced by autoantibodies from patients with neuropsychiatric symptoms, acutely modify the surface organization of NMDAR as well as partner receptors such as dopamine D1 receptors.

P32. Pooja JOSHI

Milan, Italy
pooja.joshii87@gmail.com

Microglia convert aggregated amyloid-β into neurotoxic forms through the shedding of microvesicles

Pooja Joshi[1,2], Elena Turola[1,2], Ana Ruiz[1], Alessandra Bergam[i3], Dacia Dalla Libera[3], Luisa Benussi[4], Paola Giussani[1], Giuseppe Magnani[3], Giancarlo Comi[3], Giuseppe Legname[5], Roberta Ghidoni[4], Roberto Furlan[3], Michela Matteoli[1,6]*, and Claudia Verderio[2,6]*

1. Department of Biotechnology and Translational Medicine, University of Milano
2. CNR Milano
3. INSPE Division of Neuroscience San Raffaele Scientific Institute
4. Proteomics Unit IRCCS Istituto Centro San Giovanni di Dio Fatebenefratelli
5. SISSA Department of Neuroscience
6. IRCCS Humanitas

Alzheimer’s disease (AD) is characterized by extracellular amyloid-beta deposition, which activates microglia, induces neuroinflammation and drives neurodegeneration. Recent evidence indicates that soluble pre-fibrillar Abeta species, rather than insoluble fibrils, are the most toxic forms of Abeta 1-42 preventing soluble Abeta 1-42 formation represents therefore a major goal in AD. We investigated whether membrane vesicles (MVs) produced by reactive microglia may contribute to AD neurodegeneration. We found that production of myeloid MVs, likely of microglial origin is strikingly high in mild cognitive impairment and AD patients and that AD MVs are toxic for cultured neurons. The machenism responsible for MV neurotoxicity was defined in vitro using MVs

produced from primary microglia. We demonstrated that the neurotoxicity of MVs results from i) the capability of MV lipids to promote formation of soluble Abeta species from extracellular insoluble aggregrates and ii) the presence of neurotoxic abeta s trafficked to MVs after abeta internalization into microglia. The Abeta 1-42 interacting protein PrP and anti-Abeta 1-42 antibodies could prevent soluble Abeta 1-42 binding to neurons, thereby neutralizing neurotoxicity. This study identifies microglia-derived MVs as a novel mechanism by which microglia, participate in AD degeneration, thereby suggesting new therapeutic strategies for the treatment of the disease.

P33. Haruyuki KAMIYA

Dept. Neurobiol., Hokkaido Univ. Grad. Sch. Med., Sapporo, Japan
kamiya@med.hokudai.ac.jp

Photoinactivation analysis of synaptic AMPA receptor dynamics

Haruyuki Kamiya
Dept. Neurobiol., Hokkaido Univ. Grad. Sch. Med., Sapporo, Japan

Recent studies suggested that postsynaptic expression of AMPA type glutamate receptors (AMPAR) are not static, but exchange with extrasynaptic receptors or those in intracellular reserved pools in constitutive as well as in activity-dependent manner. Several lines of evidence so far, for example fluorescence imaging of AMPAR subunits labeled with GFP as well as optical tracking of immunobeads or quantum dots labeled with specific antibodies against AMPAR subunit, support dynamic trafficking of synaptic AMPAR. However, it remains uncertain whether mode and rate of synaptic delivery of native AMPAR are similar to macromolecule-tagged or exogenously transfected ones. To reveal real-time dynamics of native AMPAR in situ, an alternative photochemical approach using a smaller compound ANQX, a photoreactive irreversible blocker of AMPAR, was adopted in mouse hippocampal slice preparations. A brief UV illumination with focal applications of ANQX to the CA1 synapses resulted in persistent suppression of excitatory postsynaptic potentials (EPSPs) for prolonged observation period up to several hours, suggesting the stable postsynaptic expression of AMPAR and minimal exchange with intracellular reserved receptors at the resting condition in intact synapses in hippocampal slices. Kinetic analysis of the timing of synaptic delivery during expression of long-term potentiation (LTP) revealed AMPAR traffic is transiently accelerated soon after LTP induction. Since this approach is readily applicable to slices obtained from genetically modifies mice, photochemical inactivation analysis using ANQX would be a powerful tool for analyzing the molecular basis of AMPAR trafficking.

P34. Rahul KAUSHIK

Magdeburg, Germany
rrajikum@lin-magdeburg.de

The synapto-nuclear messenger Jacob directly associates with the CREB complex

Rahul Kaushik[1], Anna Karpova[1], Marina Mikhaylova[1,2] and Michael R. Kreutz[1]
1. Leibniz Institute for Neurobiology, Magdeburg,
2. Department of Cell Biology, Utrecht University, The Netherlands

Activation of synaptic as well as extrasynaptic NMDARs is tightly coupled to gene expression via the transcription factor cAMP Response Element Binding Protein (CREB). Activation of synaptic NMDARs increases CREB phosphorylation and promotes the expression of neuroprotective and pro-survival genes, and the downregulation of pro-death genes. On the contrary, the activation of extrasynaptic NMDARs initiates dephosphorylation of CREB (CREB shut-off), at S133, an indispensable serine residue for its transcriptional activity, rendering it transcriptionally inactive and in turn promoting cell death. In a recent study we found that the synapto-nuclear messenger Jacob following its nuclear import can encode in the nucleus the synaptic and extrasynaptic localization of activated NMDAR (Karpova et al., 2013). The Janus face of Jacob is based on differential MAP-kinase activity and ERK-dependent phosphorylation of the serine 180 in the protein following synaptic but not extrasynaptic activation of NMDAR. We found that the neurofilament alpha-internexin associates with Jacob in a phosphorylation dependent manner and that the trimeric complex is very stable and efficiently protected against phosphatase activity. Long-distance signaling via Jacob require a larger signalosome-like protein complex and the Janus face of Jacob provides the first long-haul molecular mechanism to distinguish both NMDAR pathways in the nucleus. One might speculate that Jacob operates as a mobile hub

that docks NMDA-receptor derived signalosomes to nuclear target sites like CREB and potentially others. We therefore looked at the interaction with CREB in more detail. Over-expression of nuclear Jacob leads to CREB shut-off where as nuclear knock down of Jacob protects neurons from NMDA dependent CREB shutoff. Jacob can directly interact with the bZIP domain of CREB and Jacob binding as such will not interfere with the phosphorylation on S133 in CREB. However, Jacob might via the inetraction with the bZIP domain dock signaling molecules like ERK to the CREB complex. In line, Jacob has no significant phosphatase activity and does not dephosphorylate CREB directly but Jacob might provide an interaction interface between CREB and a CREB phosphatase.

P35. Christoph KOERBER
Heidelberg, Germany
koerber@ana.uni-heidelberg.de

Gene expression profiling of globular bushy cells during synaptic maturation

Christoph Körber[1], Anna Dondzillo[1], Gisela Eisenhardt[2], Oliver Wafzig[2], Thomas Kuner[1]
1. Institute of Anatomy and Cell Biology; Heidelberg University, D-69120 Heidelberg
2. SYGNIS Bioscience; D-69120 Heidelberg

Neurons undergo specific, cell type dependent morphological and functional changes during development. Here, we investigate how these changes are reflected in changes in the gene expression profile by using cell type specific gene expression profiling of globular bushy cells (GBCs) of the anterior ventral choclear nucleus (aVCN). GBCs give rise to the calyx of Held, a giant axosomatic synapse in the contralateral me-dial nucleus of the trapezoid body (MNTB) of the auditory brainstem, which serves as a model synapse for the investigation of synaptic release mechanisms and short-term plasticity. We perform our analysis at three distinct maturational time points: at P3, when the calyx synapse has just made contact with its target cell; at P8, a juvenile state just before the onset of hearing at ~P11; and at P21, when the synapse is fully matures. The changes in synapse morphology, from sheet-like via cup-shaped to fenestrated during this period, are paralleled by functional adjustments including e.g. decreases in action potential (AP) half-width and synaptic delay, that are optimized to enable high fidelity, high frequency synaptic transmission. Gene expression profiles of GBCs of different maturational stages were obtained by selective retrograde tracing of GBCs with fluorescently labelled Cholera toxin B (ChTx). Therefore, ChTx was stereotaxicaly injected into the rat MNTB at P2, P7 and P20. Labelled GBCs in the contralateral aVCN were harvested 24h after injection by laser mi-cro dissection and subjected to Affimetrix GeneChip profiling. The specificity of the la-belling was confirmed by recording of the firing pattern as well as reconstructing the morphology of labelled cells. Validation of microarray results was performed by two independent methods; RTqPCR for a small and nCounter assay for a large number of genes. Comparison of the expression profiles between developmental stages yielded a total of 5694 annotated genes to be regulated between any of the examined maturational changes (2689 up, 3005 down). When examining the regulated genes in detail, we found that early in development, between P3 and P8, mostly genes implicated in the general cell biology of the GBCs were regulated. Later during maturation, around the onset of hearing, the changes in synaptic function were represented in the gene expression profile with numerous voltage gated ion channels and synaptic proteins being regulated. Particular strong regulation was detected among the voltage gated ion channel and calcium binding protein genes, whereas genes involved in the SV cycle were only moderately changed.

P36. Remy KUSTERS
Eindhoven, Netherlands
r.p.t.kusters@tue.nl

Barriers in the brain: Receptor trafficking in dendritic spines

Remy Kusters, Lukas C Kapitein, Casper C Hoogenraad, Cornelis Storm
Eindhoven University of Technology, Universiteit Utrecht, Universiteit Utrecht, Eindhoven University of Technology

Dendritic spines are the small-scale neuronal protrusions at the receiving end of the signal transmission system from one neuron's axon to another neuron's dendrite. Once this connection is established, its strength is regulated, among other factors, by controlling the concentration of AMPA receptors in the spine's Post

Synaptic Density. Microscopy studies reveal a remarkable wealth of shapes among dendritic spines, and their morphology is believed to play a crucial role in the differential confinement of AMPA receptors. Presumably, this allows the spine to retain gradients in receptor density, and as a consequence its transmission strength, for long times. In this work, we present numerical and analytical results conclusively demonstrating the significant impact of shape and curvature on receptor trafficking, both passive (lateral diffusion) and active (endosomal exocytosis). We analyze the effect on diffusive processes of the morphology of dendritic spines, and show that mature morphologies called mushroom spines display a pronounced increase in the characteristic timescale for receptor escape. Moreover, the mushroom shape strongly enhances the ability of the PSD to confine receptors. Using this, we are able to show that the active recycling of AMPA receptors via exocytosis of endosomes in the spinal domain provides instantaneous and efficient control over the capture and concentration of AMPA receptors in the PSD, whereas dendritic exocytosis does not. We quantify the speed and the efficiency of this process, and again find a striking increase in the rate of receptor capturing by the PSD for mushroom spines. Taken together, our research confirms the view that mushroom serve as specific and effectively decoupled microdomains on the dendritic shaft, providing even the single dendrite with a complex palette of regulatory options. Our modeling may aid the interpretation of single-particle tracking and fluorescence recovery probes, and sheds new light on the mechanisms responsible for receptor confinement in dendritic spines.

P37. Laurent LADEPECHE

Bordeaux, France
laurent.ladepeche@u-bordeaux2.fr

Plasticity of maturing glutamate synapses requires NMDA receptors lateral mobility

Laurent Ladépêche[1#], Julien Pierre Dupuis[1#], Henrik Seth[2], Lucie Bard[1*], Juan Varela[1], Lenka Mikasova[1], Delphine Bouchet[1], Véronique Rogemond[3], Jérôme Honnorat[3], Eric Hanse[2], and Laurent Groc[1]

1. Univ. de Bordeaux, Interdisciplinary Institute For Neuroscience, UMR 5297 F-33000 Bordeaux,

2. Department of Physiology, Institute of Neuroscience and Physiology, The Sahlgrenska Academy University of Goteborg, Sweden,

3. Lyon Neuroscience Research Center INSERM U1028CNRS UMR 5292 Lyon, France

 # Equal contribution

NMDA receptors (NMDAR) play an important role in setting the plastic range of glutamate synapses, mostly through the adaptation of GluN2A- and GluN2B-NMDAR signalling. The cellular pathways by which NMDAR subtypes are dynamically trafficked during activity-dependent synaptic modifications remain however unknown. Using a combination of high-resolution single nanoparticle imaging and electrophysiological approaches in maturing hippocampal networks, we here report a NMDAR-, mGluR5-, and CAMKII-dependent rapid lateral escape of synaptic GluN2B-NMDAR occurring during long-term potentiation (LTP). Artificially preventing this surface redistribution alters the activity-dependent change in CAMKII intracellular dynamic in spines and prevents LTP. Moreover, anti-NMDAR autoimmune antibodies from patients with cognitive dysfunctions also acutely block NMDAR surface dynamics and consequently LTP. Together, these data reveal that GluN2B-NMDAR surface dynamics control the adaptation of glutamate synapses in developing neuronal networks.

P38. Capucine Le GUEN

London, United Kingdom
ucbtma2@ucl.ac.uk

Can neuromodulators affect electrical coupling between cerebellar Golgi cells?

Capucine Le Guen, R. Angus Silver
University College London

In the granular layer of the cerebellum, Golgi cells (GoCs) provide phasic and spillover mediated inhibitory input to granule cells which is thought to control spatiotemporal integration of inputs onto granule cells. The most prominent connection between GoCs is formed by dendritic electrical synapses. This electrical coupling is thought to contribute to spatial averaging in the granular layer network, and play a central role in the control of network synchrony. The conductance of electrical synapses has been shown to be regulated by

neuromodulators via pathways that affect the degree of phosphorylation of the gap junction proteins, their assembly or their turnover. This mechanism was mostly studied in the retina (NMDA receptors, dopamine), the thalamic reticular nucleus (mGluRs), the goldfish Mauthner cell (CB1, dopamine) and the hippocampus (noradrenalin). Therefore, we have investigated the possible modulation of GoCs electrical coupling by the neuromodulators present in the molecular layer. We have primarily studied the kainate and mGluRs receptors which are present both synaptically and extra-synaptically on GoCs dendrites, and can be activated by glutamate spillover from the parallel fibers of the granule cells. In addition, we have also examined the effect of common neuromodulators (nitric oxide, endocannabinoids, serotonin…).

P39. Flavie LESEPT
Caen, France
lesept@cyceron.fr

Subcellular trafficking of neuronal tPA and its potential role in synaptic plasticity

F Lesept, M Louessard, N Pasquet, L Lebouvier, Y Hommet, E Maubert, I Bardou, D Vivien
INSERM UMR-S 919 Université De Caen Basse-Normandie, Serine Proteases and Pathophysiology of The Neurovascular Unit, GIP Cycéron, 14074 Caen, France

The tissue plasminogen activator (tPA) is a serine protease initially described for its fibrinolytic properties. In addition to its vascular functions, tPA was also reported as a pleiotropic protein acting as an enzyme, a cytokine or a neuromodulator in the central nervous system. Nevertheless, its neuronal expression, trafficking and synaptic molecular influences remain largely unknown. tPA is well known to potentiate glutamatergic neurotransmission by a direct action on ionotropic receptors of N-methyl-D-aspartate (NMDAR). Briefly, tPA interacts with the GluN1 subunit of this receptor in its amino-terminal domain and thus increases NMDA-induced intracellular calcium. NMDAR regulate both the morphological maturation and the density of dendritic spines as key of the so called synaptic plasticity. Accordingly, in a model of hippocampal organotypic slices, tPA was reported to influence the mechanisms of NMDA-dependent long-term potentiation (LTP). In our present study, by using immunohistochemistry performed following colchicine exposures, we revealed a neuronal expression of tPA not only in the hippocampus but also in cortical areas (e.g. granular and agranular retrosplenial, somatosensory, piriform). Subcellular localizations and trafficking of tPA were further investigated both in vitro and in vivo following in vitro and in utero transfections of a cDNA encoding for a GFP-tPA driven by a neuronal specific promotor. Our data reveal a strong expression of tPA in the dendritic segments and a weaker expression of tPA in the axonal tract. We are now investigating the expression of tPA in pre-and postsynaptic compartments and its possible influence on the formation of new dendritic spines or/and the consolidation of matures synapses in physiological conditions.

P40. Mathieu LETELLIER
Bordeaux, France
letellier.mathieu@gmail.com

MicroRNA miR-92a regulates translation and synaptic incorporation of GluA1 containing AMPA receptors during homeostatic scaling

Mathieu Letellier*, Sara Elramah*, Magali Mondin*, Andrew Penn, Marc Landry, Daniel Choquet, Olivier Thoumine #, Alexandre Favereaux#
Univ. Bordeaux, *Interdisciplinary Institute For Neuroscience, UMR 5297, F-33000 Bordeaux, France*
CNRS, Interdisciplinary Institute For Neuroscience, UMR 5297, F-33000 Bordeaux, France
 # Equal contribution

We hypothesized that homeostatic synaptic scaling may involve local regulation of AMPA receptor translation by microRNAs in dendrites. Using bioinformatics, qRTPCR and luciferase reporter assays, we identified several brain-specific microRNAs including miR-92a, targeting the 3'-UTR of GluA1 mRNA. Immunostaining of AMPA receptors and recordings of miniature AMPA currents in primary neurons showed that miR-92a selectively regulates the synaptic incorporation of new Glua1-containing AMPA receptors during activity blockade.

P41. Jolanta LUNDGREN

Stockholm, Sweden
jolanta.lundgren@ki.se

Comparing release mechanisms for glutamate and the amyloid β-peptide from rat brain nerve terminals

Jolanta L Lundgren1, Matthew Holt2, Julia Preobraschenski3, Bengt Winblad1, Gunnar K. Gouras4, Lars O. Tjernberg1, and Susanne Frykman1*

1. Karolinska Institutet, Sweden

2. K.U. Leuven Center for Human Genetics, Belgium

3. Max Planck Institute for Biophysical Chemistry, Germany

4. Lund University, Sweden

Synaptic degeneration is one of the earliest hallmarks of Alzheimer disease. The molecular mechanism underlying this degeneration is not fully elucidated, but one key player appears to be the synaptotoxic amyloid β-peptide (Aβ). The exact localization of Aβ production and the mechanisms through which Aβ is released remain elusive. We have previously shown that Aβ can be produced in crude synaptic vesicles, and it has been reported that increased synaptic activity results in increased secreted levels of Aβ but decreased intracellular levels. Therefore, we considered whether Aβ, produced in synaptic vesicles, can be released through the same mechanisms as neurotransmitters during synaptic vesicle exocytosis. We found small amounts of Aβ were produced in pure synaptic vesicle preparations. We also studied the release of glutamate and Aβ from rat cortical nerve endings (synaptosomes) after stimulation by KCl or 4-aminopyridine. Glutamate release was measured by conversion of NADP to NADPH by glutamate dehydrogenase, and Aβ release was determined by using ELISA. Preliminary results show that whereas glutamate release was completely activity-dependent, a considerable part of the Aβ release occurred without stimuli. Thus, at least part of the secreted Aβ is released through a mechanism distinct from synaptic vesicle exocytosis.

P42. Romain LY - *Cancelled registration*

Paris France
rly@biologie.ens.fr

T-type Channel Blockade Impairs Parallel fiber – Purkinje cell Long-Term Potentiation and Cerebellar Learning

Romain Ly, Guy Bouvier, Martijn Schonewille, Arnaud Arabo, Laure Rondi-Reig, Clément Lena, Mariano Casado, Chris I. De Zeeuw, and Anne Feltz

1. IBENS École Normale Supérieure CNRS UMR 8197 F-75005-Paris, Dept of Neuroscience ErasmusMC NL-Rotterdam, UFR Sciences Rouen F-76821 Mont Saint Aignan, UPMC UMR 7102, F-75005 Paris

CaV3.1 T-type channels are abundant at the cerebellar synapse between parallel fibers (PFs) and Purkinje cells (PCs) where they contribute to synaptic depolarization. So far, no specific physiological function has been attributed to these channels neither as charge carriers nor more specifically as Ca2+ carriers. Here we analyze their incidence on synaptic plasticity, motor behavior and cerebellar motor learning comparing WT animals and mice where T-type channel function has been abolished either by gene deletion or by acute pharmacological blockade with TTA-P2 (Merck and Co, Inc). At the cellular level, we show that CaV3.1 channels are required for long-term potentiation (LTP) at PF-PC synapses whereas their blockade has no effect on long-term depression (LTD). Moreover, basal simple spike discharge of PC in KO mice is modified. Acute or chronic T-type current blockade results in impaired motor performance in particular when a good body balance is required. Since motor behavior integrates reflexes and past memories of learned behavior, this suggests impaired learning. Indeed, subjecting the KO mice to a vestibulo-ocular reflex (VOR) phase reversal test reveals impaired cerebellum-dependent motor learning. These data identify a role of low-voltage activated calcium channels in synaptic plasticity and establish a role for CaV3.1 channels in cerebellar learning.

P43. Vincent MAINGRET

Bordeaux, France
vincent.maingret@gmail.com

PGE2 modulates synaptic plasticity at hippocampal mossy fiber - CA3 synapse

Vincent Maingret[1,2], Christophe Mulle[1,2] and Thierry Amédée[1,2]
1. Univ. Bordeaux, IINS, UMR 5297, F-33000 Bordeaux, France
2. CNRS, IINS, UMR 5297, F-33000 Bordeaux, France

There are considerable evidences that neuroinflammation and Alzheimer's disease (AD) are intimately related. Among different inflammatory molecules highly expressed in the course of the pathology, prostaglandin E2 (PGE2) may play a key role.Interestingly PGE2 has recently emerged as a potent modulator of synaptic plasticity in the hippocampus and numerous studies have lead to the hypothesis that the early stage of AD was caracterized by hippocampal synaptic dysfunction. PGE2 acts on four G protein coupled receptors (EP1R-EP4R) subtypes. At the canonical Schaffer collateral - CA1 synapse, PGE2 modulates basal synaptic transmission and plasticity such as long term potentiation (LTP). However the effect of PGE2 has not been studied at the mossy fiber (Mf) pathway which connects dentate gyrus granule cells to CA3 pyramidal neurons. Remarkably Mf-CA3 LTP is presynaptic, independant of NMDA receptors and due to an increase of probability of neurotransmitter release.In this study we investigated the effect of acute application of PGE2 on presynaptic plasticity at the Mf-CA3 synapse. PGE2 (10 µM) and either ONO-AE1-249-01 (1 µM), a specific agonist of EP2R, or sulprostone (1 µM), a potent agonist of EP3R, had no effect on basal synaptic transmission. On contrast, PGE2 (10 µM) or sulprostone (1 µM) strongly impaired Mf-CA3 LTP and those effects were blocked by the co-application of a specific antagonist of EP3R (ONO-AE3-240, 1 µM).EP3R is negatively coupled to cAMP production through G-proteins, therefore our data suggest that PGE2 by altering presynaptic level of cAMP and PKA activity decreases the release probability of neurotransmitter. Next experiments will investigate if EP3R is involved in synaptic dysfunction at the Mf-CA3 synapse in a transgenic model of AD-like pathology, the APP/PS1 mouse.

P44. Manuel MAMELI

Paris, France
manuel.mameli@inserm.fr

Drug-evoked synaptic plasticity in the lateral habenula a substrate for aversive states

Matthieu Maroteaux, Manuel Mameli
Institut du fer à moulin INSERM UMR S 839, Université Pierre et Marie Curie, Paris

Addictive drugs increase dopamine (DA) levels and trigger synaptic adaptations in the mesocorticolimbic system. Neurons located in the lateral habenula (LHb) modulate the activity of DA neurons, DA release and adaptively tune goal-directed behaviors. We put forward the hypothesis that adaptations in the LHb may represent an intermediate cellular substrate for drug taking underlying either rewarding or aversive properties of drugs. To this end we combined retrograde tracing and ex-vivo patch-clamp recordings to assess the influence of cocaine experience on excitatory transmission onto subsets of LHb neurons. We find that cocaine selectively strengthens glutamatergic synapses onto LHb neurons sending axons to the rostromedial tegmental nucleus, a GABAergic structure linking the LHb with DA neurons of the ventral tegmental area. Furthermore, cocaine-evoked synaptic strengthening, while inducing a postsynaptic accumulation of AMPA receptors, does not modify subunit composition or single-channel conductance. This synaptic potentiation unmasks a long-lasting AMPA receptor-dependent synaptic potentiation upon a stimulation protocol pairing presynaptic glutamate release with somatic hyperpolarization. This results unravels an early, projection-specific, cocaine-evoked synaptic potentiation in the LHb that may represent a key step for the functional reorganization of the mesolimbic system after drug exposure.

P45. Wilfrid MAZIER

Alicante, Spain
wmazier@hotmail.fr

GluK1 overexpression in a mouse model of Down syndrome results in an increase of inhibitory synaptic transmission

Mazier W, Paternain AV, and Lerma J
Instituto de Neurociencias de Alicante (CSIC-UMH)

Kainate receptors belong to one subfamily of the glutamatergic receptors. This receptor family are composed by homo- or heterodimers of five different types of subunits named GluK1 to GluK5. The human gene coding for the kainate receptor subunit GluK1 (GRIK1) is located on the chromosome 21 that triplicates in Down syndrome. In animal models of this disease, Grik1 is situated on the short triplicated segment. Previous work on trisomic models has found an imbalance between excitatory and inhibitory hippocampal synaptic activity.In this study, we used Ts2Cje mouse model to investigate a putative link between a GluK1 excess of function and altered synaptic properties in the hippocampus of trisomic mice. Trisomic animals were differentiated from their diploid littermates (DLM) by multiplex qPCR. RTqPCR analysis revealed that Grik1 mRNA levels are increased by more than 50% in different structures of trisomic brain. Despite the lack of GluK1 specific antibody, we found in electrophysiological experiments that the extra gene-copy produces an increase of GluK1 functional protein at the membrane of trisomic cells. Hippocampal patch clamp recordings from CA1 brought out significant increase in basal and evoked inhibitory drive onto pyramidal cells and interneurons from trisomic mice. Ts2Cje animals also presented an impaired of long term potentiation in hippocampal CA1 pyramidal cells. We also observed that GluK1 selective drugs may rescue the over-inhibition phenotype observed in trisomic mice. Our data support the implication of GluK1-containing kainate receptors in the control of network activity in the area CA1 of the hippocampus which is altered in Down syndrome. Further molecular and pharmacological experiments will reveal more details about the effect of GluK1 overexpression on synaptic physiology and pathophysiology of this disease.

P46. Zofia MIJAKOWSKA

Warsaw, Poland
zmijakow@nencki.gov.pl

αCaMKII-autophosphorylation in the development of addiction

Zofia Mijakowska, Szymon Łęski, Błażej Ruszczycki, Paulina Leśniewska, Kasia Radwańska
Nencki Intitute of Experimental Biology, PAS, Warsaw, Poland

Alcohol causes numerous changes in the nervous system, among which dendritic spine morphology alterations have just begun to be studied. αCaMKII-autophosphorylation has been shown to regulate spine morphology in vitro. We provide evidence that after chronic alcohol exposure wild-type mice develop structural changes in dendritic spines in dentate gyrus that are different from the changes observed in αCaMKII-autophosphorylation-deficient mice (T286A mutants). Those changes correlate with addiction-like behavior. We also found that T286A mice are less prone to extreme behavior; they show neither very high motivation for alcohol nor drastic avoidance. Thus our data suggest that αCaMKII-autophosphorylation is involved in the development of alcohol addiction-related behavior as well as homeostatic control of dendritic morphology.

P47. Nikolaos MITAKIDIS

Oxford, United Kingdom
nikos@strubi.ox.ac.uk

Proteoglycan regulation of RPTPσ-TrkC mediated synaptogenesis

Nikolaos Mitakidis, A. Radu Aricescu
Division of Structural Biology, Wellcome Trust Centre for Human Genetics, University of Oxford, Roosevelt Drive, Oxford, OX3 7BN, UK

Receptor protein tyrosine phosphatases (RPTPs) are type IIa cell surface receptors involved in neuronal growth and guidance, as well as excitatory synapse formation. Extracellular regions of RPTPs have been shown to interact, via a shared binding site, with a diverse range of heparan sulfate proteoglycans (HSPGs) and chondroitin sulfate proteoglycans (CSPGs). Such events have been previously shown to act as switches in RPTP-mediated signaling, leading to different neuronal extension outcomes [1]. RPTPσ has been reported to interact with a postsynaptic neurotrophin receptor tyrosine kinase (Trk), TrkC, facilitating bidirectional adhesion and excitatory synaptic organization [2]. Furthermore, action potentials are exhibited by these excitatory synapses, only upon release of HSPGs from surrounding glia cells [3]. We hypothesise that while axon growth is promoted by RPTPσ-HSPGs interactions, TrkC is a tag for the synaptic targets, where the competition with proteoglycans switches growth to differentiation of a pre/post synaptic apparatus. Crystallographic snapshots of the RPTPσ-TrkC complex have been determined and showed an overlap of the proteoglycan and TrkC binding sites on RPTPσ. Surface plasmon resonance experiments with structure-based mutants and wild-type proteins were performed to characterise the interaction between RPTPσ-TrkC, as well as the competition between TrkC and proteoglycans for RPTP binding. A series of structure-based mutants were tested in co-culture cellular settings, in order to assess the role of these interactions on the initiation of synapse formation. Studying this trans-synaptic interaction assists in deducing a prototypical molecular mechanism which describes how extending axons recognise and mark cellular targets for subsequent synapse establishment. It also paves the way for future studies that will address the activity-dependent control of this bi-directional signal transduction across the postsynaptic membrane.

[1] Coles CH, Shen Y, Tenney AP, Siebold C, Sutton GC, Lu W, Gallagher JT, Jones EY, Flanagan JG, Aricescu AR. Proteoglycan-specific molecular switch for RPTPσ clustering and neuronal extension. Science, 332:484-488 (2011).
[2] Takahashi H, Arstikaitis P, Prasad T, Bartlett TE, Wang YT, Murphy TH, Craig AM. Postsynaptic TrkC and presynaptic PTPσ function as a bidirectional excitatory synaptic organizing complex. Neuron, 69:287-303 (2011).
[3] Allen NJ, Bennett ML, Foo LC, Wang GX, Chakraborty C, Smith SJ, Barres BA. Astrocyte glypicans 4 and 6 promote formation of excitatory synapses via GluA1 AMPA receptors. Nature, 486: 410-414 (2012).

P48. Maïté MOREAU

Bordeaux, France
maite.moreau@inserm.fr

Short-term and long-term memory deficits in Looptail heterozytote mice mutated for the planar polarity protein Vangl2

Maïté M. Moreau[1,2], Benjamin Robert[1,2], Chantal Medina[1,2], Alice Al Abed[2,3], Laurent Braya-Bruno[2,3], Aline Marighetto[2,3]*, Mireille Montcouquiol[1,2]* & Nathalie Sans[1,2]
1. INSERM, Planar Cell Polarity Group, Neurocentre Magendie, Unité U862, Physiopathologie de la plasticité neuronale, U862, F-33000 Bordeaux, France
2. Univ. Bordeaux, Neurocentre Magendie, Physiopathologie de la plasticité neuronale, U862, F-33000 Bordeaux, France
3. INSERM, Pathophysiology of declarative memory Group, Neurocentre Magendie, Unité U862, Physiopathologie de la plasticité neuronale, U862, F-33000 Bordeaux, France

Abnormalities in brain structures or improper formation and maintenance of synapses impact on learning, memory, decision-making and in general adaptative behaviour. In recent years, a growing amount of data have implicated Planar Cell Polarity (PCP) signaling in various mechanisms shaping the brain including neuronal migration, neuronal polarity, axonal guidance, dendrite morphogenesis and synaptogenesis, to cite a few. vangl2 is a highly conserved gene throughout evolution, which code for Vangl2, one of a central protein in PCP signaling. We used the Looptail mice, which carry a point mutation in Vangl2 that causes a serine-to-asparagine transition at aa position 646 (Vangl2Lp) to evaluate the impact of PCP signaling in adult mice brain shape and function. Using 3D reconstruction of the brain, we report that a heterozygous mutation for vangl2 in looptail mutant mice (Vangl2Lp/+) leads to an overall reduction of the brain size associated to specific structure or tract volume changes. For instance, we observed a 15 % reduction of the hippocampus volume, including the dentate gyrus. Moreover, we observed an absence of hippocampal commissures as well a severe reduction of the corpus callosum. Since incomplete callosal agenesis or hippocampal commissures have been associated with cognitive impairments, we performed a behavioral and morphological characterization of the Vangl2Lp/+ mice. Using the radial-maze and different memory tasks, we found that Vangl2Lp/+ mice displayed significant

impaired memory flexibility in the long-term relational memory task, and short-term working memory organization disruption. Altogether, our data suggest that Vangl2 plays an important role in the 3D organization of brain structures, and that its mutation leads to memory consolidation defects.

P49. Edoardo MORETTO

Milan, Italy
edoardo.moretto@studio.unibo.it

TSPAN5 protein in the brain: a new regulator of GluA2 trafficking

Moretto E, Zapata J, and Passafaro M
CNR Institute of Neuroscience, Department BIOMETRA, University of Milan, Milan, Italy

The Tetraspanin-5 (TSPAN5) protein is a member of the tetraspanin superfamily, a group of transmembrane proteins with several functions that span from cell-cell adhesion to signals transduction and some of them have been shown to have fundamental role in the correct development of mammals' nervous system. TSPAN5 is ubiquitously expressed and conserved in mammals and its messenger RNA reaches the maximum expression in the nervous system. This background moves us to investigate the exact localization and function of this protein in the brain. We have characterized the expression and localization of TSPAN5 protein finding high levels of protein in the postsynaptic compartment as shown by co-localisation with postsynaptic proteins in immunofluorescence analysis on rat hippocampal cultured neurons and by enrichment of the protein in the postsynaptic density fraction obtained from adult rat brain. The silencing through shRNA approach in cultured neurons leads to a dramatic decrease in the number of dendritic spines and synapses together with a decrease of the main synaptic proteins. We identified AP4σ as a new interactor of the intracellular C-terminal tail of TSPAN5 through a yeast two-hybrid screening and co-immunoprecipitation experiments. AP4σ is a subunit of the AP4 adaptor complex that has been connected to Ionotropic Glutamate Receptor (GluA) sorting to the somatodendritic compartment through the interaction with TARPs. Preliminary results show the existence of TSPAN5-AP4-GluA2 complex in rat brain and we observed in TSPAN5 silenced neurons an accumulation of AP4 in the soma and a reduction of both global and surface level of GluA2. This reduction could be rescued by the blockage of the lysosomal degradation pathway suggesting a role of TSPAN5 in the internalization or in the insertion of GluA2 in the membrane. We are currently investigating the exact mechanism by which TSPAN5 acts on GluA2 and thus on synapse formation.

P50. Stefano MUSARDO

Milan, Italy
stefano.musardo@unimi.it

ADAM10 exocytosis/endocytosis in spines: role in Alzheimer's disease

Stefano Musardo, Elena Marcello, Claudia Saraceno, Silvia Pelucchi, Daniele Di Marino, Barbara Borroni, Anna Tramontano, Isabel Pérez-Otaño, Alessandro Padovani, Fabrizio Gardoni, and Monica Di Luca
Università degli Studi di Milano, Dipartimento di Scienze Farmacologiche e Biomolecolari and Centre of Excellence on Neurodegenerative Diseases, Milan, Italy

Alzheimer's disease (AD) is the most common neurodegenerative disorder characterized by progressive loss of synapses and neurons and accumulation of insoluble deposits of amyloid beta-peptide (Abeta). Although AD is emerging as the most prevalent and socially disruptive illness of aging populations, it is currently incurable.Abeta derives from the amyloid precursor protein (APP), which can follow 2 mutually exclusive pathways in the cell. The amyloidogenic pathway involves BACE and gamma secretase activities and leads to Abeta formation. On the other hand, the main protagonist of the non-amyloidogenic pathway is ADAM10, a disintegrin and metalloproteinase 10, which cleaves APP in the domain corresponding to Abeta, thus precluding Abeta production. ADAM10 cleaves its substrates when correctly localized at the plasma membrane, therefore the mechanisms regulating its trafficking can affect its activity. Since the modulation of ADAM10 synaptic localization through ADAM10 membrane insertion/removal could constitute an innovative therapeutic strategy to finely tune its shedding activity, we have investigated the mechanisms underlying ADAM10 endocytosis. We show that ADAM10 removal from the plasma membrane is mediated by clathrin-dependent endocytosis and we describe the clathrin adaptor AP2, a heterotetrameric assembly which initiates the endocytosis process, as new interacting partner of ADAM10 C-terminal domain. In particular, we identify an

atypical binding motif for AP2 complex in ADAM10 cytoplasmic tail, which is relevant for ADAM10 endocytosis and the modulation of its plasma membrane levels. Moreover, we describe a pathological alteration of ADAM10/AP2 association in AD and a physiological role in activity-dependent synaptic plasticity. We demonstrate that long-term potentiation induces ADAM10 endocytosis, through AP2 association, and decreases surface ADAM10 levels and activity, while long-term depression promotes ADAM10 synaptic membrane insertion and stimulates its activity.Finally we have designed different cell permeable peptides able to interfere with ADAM10/AP2 association and, thereby, to reduce ADAM10 endocytosis.

P51. Nael NADIF KASRI
Nijmegen, Netherlands
n.nadif@donders.ru.nl

A critical role for ARHGAP12 in regulating excitatory synaptic structure and function

Wei Ba, Jori Van Der Raadt, Astrid Oudakker, Astrid Valles, Hans Van Bokhoven, and Nael Nadif Kasri
Radboud University Nijmegen Medical Centre, Donders Institute For Brain Cognition, and Behaviour Department of Cognitive Neuroscience, 6500 HB Nijmegen, The Netherlands

Activity-dependent changes in the strength of excitatory synapses are thought to be key cellular mechanisms that contribute to the plasticity of neuronal networks underlying learning and memory. Key mechanisms for the regulation of synaptic strength are the dynamic change in size and number of dendritic spines and the synaptic incorporation and removal of AMPA-type glutamate receptors (AMPAr). As key regulators of the actin cytoskeleton the Rho subfamily of GTP-binding proteins play a critical role in synaptic development and plasticity. They shuttle between the active GTP- bound form and the inactive GDP- bound form under the regulation of dedicated guanine nucleotide exchange factors (GEFs) and GTPase-activating proteins (GAPs). More than 80 human GEFs and GAPs have been identified, most of which are expressed in the brain with a specific spatial and temporal expression pattern. However, the function of most GEFs and GAPs in the brain has not been elucidated. Here, we report the functional characterization of ARHGAP12, a RhoGAP that negatively regulates Rac1 signaling. ARHGAP12 is specifically expressed in the CA1 region of the hippocampus and its expression decreases after the first postnatal week, coinciding with the maturation process of excitatory synapses. At the cellular level ARHGAP12 localizes to the postsynaptic site of mature excitatory synapses as it co-localizes with PSD-95. Overexpression of ARHGAP12 in hippocampal neurons reduces the number of excitatory synapses and dramatically decreases the frequency and amplitude of AMPAr-mediated miniature excitatory postsynaptic currents. Conversely downregulation of Arhgap12 rapidly converted silent synapses to active synapses, therefore enhancing AMPAr-mediated excitatory synaptic transmission and increasing the proportion of mature spines. Together these data suggest that endogenous ARHGAP12 functions as a synaptic break during development and bidirectionally regulates excitatory synaptic strength. Interestingly, Arhgap12 was recently identified as target of Fragile X mental retardation protein. In accordance we found that ARHGAP12 is rapidly translated in response to metabotropic glutamate receptor (mGluR) activation in mature neurons. Finally, Arhgap12 knockdown impeded mGluR-mediated log-term depression in hippocampal brain slices. Together our data show that ARHGAP12 is a novel synaptic RhoGAP that plays a critical role in regulating excitatory synaptic structure and function.

P52. Valentin NÄGERL
Bordeaux, France
valentin.nagerl@u-bordeaux2.fr

Microglia-synapse interactions during synaptic plasticity

Thomas Pfeiffer, Elena Avignone, U. Valentin Nägerl
Interdisciplinary Institute for Neuroscience, UMR 5297 CNRS / Université Bordeaux Segalen

Activity-dependent modifications of neuronal synapses are a hallmark of the mammalian brain, underlying the development and wiring of neural circuits and higher brain functions such as learning and memory. While cellular memory processes and synaptic plasticity have traditionally been studied from a neuronal point of view, recent work indicates that glia cells such as astrocytes and microglia can substantially influence the function and regulation of synapses. Besides their role in pathology, surprising effects in the healthy brain have

recently been described for microglia, e.g. on synapse pruning during development and synaptic transmission, raising the question whether microglia are involved in the morpho-functional remodeling of synaptic connections. To address this hypothesis we set out to investigate the morphological interactions between microglia and synapses during synaptic plasticity. We performed time-lapse 2-photon imaging together with electrophysiological recordings in acute hippocampal slices obtained from transgenic mice, where microglia and neurons are robustly labeled with GFP and YFP, respectively. While inducing LTP at CA3-CA1 synapses, we recorded the dynamic interactions between microglial processes and dendrites of CA1 pyramidal neurons. The main finding is that LTP induction leads to an increase in the motility of microglia processes and changes in their dynamic interactions with synapses, increasing the duration while decreasing the frequency of contacts formed with CA1 dendrites. These effects were blocked by the NMDAR blocker D-APV. Our study establishes that the morphological dynamics of microglia and their interactions with dendrites are significantly impacted during LTP, indicating that microglial processes become more intimately engaged with synapses during synaptic plasticity. These observations warrant further investigations into the signaling mechanisms and functional impact of microglia-synapse crosstalk during synaptic plasticity.

P53. Richard ORTEGA
Gradignan, Bordeaux
ortega@cenbg.in2p3.fr

Iron storage within dopamine vesicles revealed by synchrotron radiation X-ray fluorescence nano-imaging

Carmona Asuncion, Ortega Richard
Centre d'études nucléaires de Bordeaux Gradignan, CNRS, Bordeaux University, Gradignan, France

The detection of trace elements at the subcellular level is a challenging task that requires sophisticated analytical developments. In this study, we report how chemical element imaging was performed in subcellular compartments of dopaminergic cells at high spatial resolution using the x-ray fluorescence nanoprobe developed at the European Synchrotron Radiation Facility. High spatial resolution is obtained using the concept of a secondary source focused to a 90 nm probe by multilayer mirrors bent in Kirkpatrick-Baez geometry. This original setup was applied for trace metals mapping of single dopaminergic cells. This cellular model is able to differentiate upon exposure to nerve growth factor and to extend neurite-like processes. Two important results were obtained. First, iron accumulates into dopamine vesicles, found mainly in primary neurite outgrowths and distal ends (Ortega et al., 2007). Second, thin neurite-like processes produced by differentiated cells accumulate copper, zinc, and to a minor extent lead (Carmona et al., 2008). Overall, the high resolution imaging of single neuron-like cells offers unique information to understand the role of trace metals in neurochemistry (see also Carmona et al., 2010; Kosior et al., 2012).

Carmona A., Cloetens P., Devès G., Bohic S., Ortega R. (2008) Nano-imaging of trace metals by synchrotron X-ray fluorescence into dopaminergic single cells and neurite-like processes. Journal of Analytical Atomic Spectrometry, 23, 1083-1088
Carmona A., Devès G., Roudeau S., Cloetens P., Bohic S., Ortega R. (2010) Manganese accumulates within Golgi apparatus in dopaminergic cells as revealed by synchrotron X-Ray fluorescence nano-imaging. ACS Chemical Neurosciences, 1, 194-203
Kosior E., Bohic S., Suhonen H., Ortega R., Devès G., Carmona A., Marchi F., Guillet J.F., Cloetens P. (2012) Combined use of hard X-ray phase contrast imaging and X-ray fluorescence microscopy for subcellular metal quantification, Journal of Structural Biology, 177, 239-247
Ortega R., Cloetens P., Devès G., Carmona A., Bohic S. (2007) Iron storage in neurovesicles revealed by chemical nano-imaging. PLoS ONE, 2(9), e925

P54. Aude PANATIER
Bruges, France
aude.panatier@inserm.fr

Nanoscale imaging of the tripartite synapse morphology using STED microscopy

A Panatier[1,2,3], SHR Oliet[1,2], UV Nägerl[1,3]
1. Univ. Bordeaux, IINS, UMR5297, F-33000 Bordeaux, France,

2. INSERM U862, Neurocentre Magendie, F-33000 Bordeaux, France
3. CNRS, IINS, UMR 5297, F-33000 Bordeaux, France

Research on astrocytes in the brain has in recent years led to a thorough reappraisal of their role in brain function, extending greatly beyond the classic view as mere providers of structural support to neurons. Indeed, the concept of the tripartite synapse, composed of neuronal and astrocytic elements, recognizes the important role of these glial cells in the regulation of information transfer at synapses in the central nervous system, in a highly dynamic and multifaceted way. Despite much progress, a major obstacle for investigating the crosstalk between astrocytes and neurons at synapses is their enormous morphological complexity and small size. This is particularly true for secondary and tertiary astrocytic processes, which typically are much too small to be properly resolved by conventional light microscopy.To overcome this limitation and to better understand how astrocytes interact with hippocampal glutamatergic synapses, we used stimulated emission depletion (STED) microscopy in organotypic hippocampal slices. We imaged neurons and astrocytes in two colors using a home-built STED microscope, which has a spatial resolution of at least 70 nm. To visualize astrocytic and neuronal morphology in the same tissue we used patch pipettes to fill astrocytes with Alexa Fluor 488 dye and to record from them electrophysiologcially in brain slices from transgenic Thy1-YFPH mice. Our experiments demonstrate that it is possible to visualize the complex and hyperfine morphology of astrocytes by STED microscopy, faithfully resolving astrocytic processes that are much too small for conventional light microscopy. Importantly, the two-color STED approach allows us to reveal and investigate the intimate morphological relationship between spines and astrocytic processes embedded in living brain slices.

P55. Andrew PENN

Bordeaux, France
andy.c.penn@gmail.com

Cross-linking AMPA receptors and synaptic physiology in intact tissue

Penn AC[1,2], Carbone AL[3], Espana A[1,2], Royer L[1,2], Breillat C[1,2], Groc L[1,2], Plested A[3], and Choquet D[1,2]
1. Univ. Bordeaux, Institut interdisciplinaire de neurosciences, UMR5297, F-33000 Bordeaux, France
2. CNRS, Institut interdisciplinaire de neurosciences, UMR 5297, F-33000 Bordeaux, France
3. Leibniz-Institut für Molekulare Pharmakologie (FMP), Robert-Roessle-Strasse 10, 13125 Berlin, Germany & NeuroCure, Charité-Universitätsmedizin, Berlin, Charitéplatz 1, 10117 Berlin, Germany

AMPA receptors (AMPARs) at the synapse are in dynamic equilibrium with extrasynaptic receptors. Disrupting this dynamic by antibody-mediated cross-link impacts AMPAR-mediated short-term plasticity (STP) in cultured hippocampal neurons (Heine et al., 2008, Science). Specifically, these experiments proposed that the rapid exchange of receptors with an extrasynaptic reservoir helps to replenish the post-synaptic density (PSD) with non-desensitized receptors. This could be significant given that many factors modulate receptor diffusion (Opazo et al., (2012) Curr Opin Neurobiol). However, the impact of receptor dynamics at synapses in intact tissue remains contraversial for two reasons: 1) synapses in slices typically have a low transmitter release probability; and 2) the extracellular matrix is assumed to be more extensive in intact tissue (Frischknecht et al., 2009, Nat Neurosci; Kochlamazashvili et al., 2010, Neuron). These factors could potentially render receptor desensitization irrelevant and AMPAR exchange too slow in any case. Cross-linking in intact tissue is difficult because of the large molecular weight of whole IgG antibodies. Therefore, we have devised and tested a strategy to express and acutely cross-link biotinylated recombinant receptors with tetravalent biotin-binding proteins in intact brain tissue, which were prepared either acutely or by organotypic culture. Our results strikingly show that receptor exchange is abundant at spines in slices and that robust effects on paired-pulse responses can be observed on both EPSCs and EPSPs. Since we rule out any non-specific effect of cross-linking on STP or direct effects of cross-linking on receptor biophysics, we conclude that receptor diffusion can boost facilitation and information processing at synapses in brain slices.

P56. David PERRAIS

Bordeaux, France
david.perrais@u-bordeaux2.fr

Endocytosis and recycling of receptors in neuronal dendrites

Damien Jullié[1,2], Daniel Choquet[1,2], David Perrais[1,2]
1. Univ. Bordeaux, Institut interdisciplinaire de neurosciences , UMR5297, F-33000 Bordeaux, France
2. CNRS, Institut interdisciplinaire de neurosciences, UMR 5297, F-33000 Bordeaux, France

Receptor recycling is crucial for many aspects of cellular physiology. In neurons, it has a central role in receptor signalling, in the establishment of neuronal polarity and in synaptic plasticity. We have studied several aspects of receptor endocytosis, formation of recycling endosomes (REs) and RE exocytosis using live fluorescence imaging of cultured hippocampal neurons. To follow receptors in their intracellular journey, we have transfected neurons with receptors tagged with superecliptic phluorin (SEP), a fluorescent marker which is visible at neutral pH and not at the acidic pH of endosomes, and we have incubated these neurons with extracellular, pH insensitive, red fluorescent ligands, which will be internalised with receptors. In the case of β2 adrenergic receptors (SEP-β2AR), we observe after application of an agonist (isoproterenol 10 µM) a strong internalization and the formation of clusters of receptors corresponding to REs. After a few minutes, we observe single exocytosis events, i.e. localized bursts of SEP-β2AR fluorescence, at individual endosomes. We frequently observe multiple exocytosis events at single endosomes, suggesting that REs can undergo several rounds of exocytosis. Indeed, we observe two types of exocytosis events: discharge events where receptors rapidly diffuse into the plasma membrane, and display events where receptors remain clustered after exocytosis. Using fast extracellular solution changes, we show that display events correspond to transient opening of a fusion pore (“kiss-and-run”) with a median opening time of 2.6 s. Moreover, we show that this behaviour is found for several recycled receptors, transferrin receptor (TfR) and the glutamate receptor GluA1. Finally, we find that the RE marker Rab11 remains enriched after display exocytosis, is selectively discarded shortly after discharge exocytosis, and controls RE shape and ability to provide receptors at the surface. Therefore, neurons can regulate the exocytosis of REs locally and rapidly for the delivery of receptors at the plasma membrane.

P57. Bérangère PINAN-LUCARRE

Villeurbanne, France
pinan@biologie.ens.fr

An ADAMTS-Like protein organizes post-synaptic domains in C. elegans

Berangere Pinan-Lucarre, Marie Pierron, Haijun Tu, Jean-Louis Bessereau
CGPhiMC CNRS UMR5534 Universite Lyon 1

The formation of chemical synapses requires the coordinated differentiation of microdomains to cluster post-synaptic receptors precisely in front of neurotransmitter release sites. We use the C. elegans excitatory and inhibitory neuromuscular junctions (NMJ) as a model system to decipher novel molecular mechanisms controlling the paired differentiation of pre- and post-synaptic domains. By genetic screening, we identified Ce-Punctin, an evolutionarily conserved ADAMTS-Like protein, as a novel synaptic organizer that triggers the synaptic clustering of post-synaptic receptors. Loss of Ce-Punctin triggers a fragmentation and a spectacular redistribution of both excitatory and inhibitory clusters of receptors at extra-synaptic areas of muscle cells, while pre-synaptic organization is not altered. The function of Ce-Punctin reveals that pre- and post-synaptic domain differentiation can be genetically uncoupled in this system. In addition, this is the first report of a synaptogenic role of an ADAMTS-Like protein that may be conserved in the mammalian central nervous system.

P58. Vera PINHEIRO

Bordeaux, France
vera.pinheiro@inserm.fr

The autism associated Planar Cell Polarity protein Scribble1 regulates AMPA receptor-dependent synaptic plasticity trafficking

Pinheiro VL[1,2], Piguel NH[1,2], Hosi E[3,4], Blanc JM[5,6], Hilal M[1,2], Moreau MM[1,2], Medina C[1,2], Breillat C[3,4], Vuillard L[5], Choquet D[3,4], Montcouquiol M[1,2], & Sans N[1,2].
1. INSERM, Planar Cell Polarity Group, Neurocentre Magendie, Unité U862, Physiopathologie de la plasticité neuronale, U862, F-33000 Bordeaux, France.

2. Univ. Bordeaux, Neurocentre Magendie, Physiopathologie de la plasticité neuronale, U862, F-33000 Bordeaux, France.
3. CNRS, Institut interdisciplinaire de neurosciences, UMR 5297, F-33000 Bordeaux, France

The appropriate trafficking of AMPA subtype of glutamate receptors in and out of synapses is crucial for different forms of synaptic plasticity that trigger processes such as memory formation, consolidation or retrieval. In hippocampal pyramidal neurons, the induction of LTP increases the delivery of GluA1 to membrane through a mechanism that requires its PDZ binding domain. SCRIB is a candidate risk gene for autism spectrum disorders, which code for Scrib1, a leucine-rich repeats and 4 PDZ domains containing protein implicated in brain development and plasticity. We have shown previsously that Scrib1 controls the number of synaptic NMDAR. Here, we show that Scrib1 forms a PDZ-dependent macromolecular complex with TARPs to regulate AMPAR. In hippocampal neurons Scrib1 specifically modulates GluA1 internalization and recycling under basal conditions but not GluA2. We also used the superresolution imaging technique, uPAINT, to show that Scrib1 does not influence endogenous GluA2 containing receptor's local diffusion under basal conditions. However, using live imaging with SEPGluA constructs, we showed that Scrib1 critically drives NMDA-dependent traffic of both GluA1 and GluA2 subunits. Actin dynamics play a critical role in endocytosis and exocytosis processes. We have shown previously that Scrib1 co-localizes with filamentous (F)-actin in Cos7 cells. Mutating potential actin or Arp2/3 binding sites on Scrib1 prevent such co-localization, potentially interfering with AMPAR traffic. Taken together, our findings define a molecular mechanism by which the autism associated protein Scribble1 controls AMPAR post-endocytic traffic but not their lateral mobility and provide a new insight into the fundamental aspects of synaptic plasticity.

P59. Maria Joana PINTO

Vila do Bispo, Portugal
mjoanagp@gmail.com

Proteasome as a local regulator of presynapse assembly

Maria Joana Pinto[1,2], Ramiro D Almeida[1]
1. Center For Neuroscience and Cell Biology, University of Coimbra, Portugal
2. PhD Programme In Experimental Biology and Biomedicine (PDBEB), Center for Neuroscience and Cell Biology, University of Coimbra, Portugal

Differentiation of the presynaptic terminal is known to rely on target-derived factors that promote the assembly of the active zone and clustering of synaptic vesicles. This is a highly rapid and dynamic event, which occurs in a time-scale of minutes to few hours. Furthermore, axons are extremely long and presynaptic terminals are to be formed in remote sites. Under these circumstances, it is reasonable to believe that axons rely on local mechanisms to support and sustain their prompt response to cues, which will then lead to a site-specific clustering of presynaptic components. Local control of protein turnover is rapidly gaining acceptance as an on-site event involved in synapse formation, and in fact, local downregulation of synaptically localized kinases has been shown to induce presynaptogenesis[1-3]. Moreover, the proteasome was shown to be spatially and temporally recruited to spines, thus providing a mechanism for site-specific control of protein composition[4]. However, the mechanisms by which the proteasome regulates presynaptic structures are poorly understood. Here we show that presynaptogenesis induces local proteasome recruitment. We observed that FGF22, a presynaptic organizer, increases the number of Rpt3 clusters with a temporal pattern similar to the formation of new synapses. Moreover, FGF22 induced the recruitment of proteasomes to synaptic sites and local protein degradation. In line with these results, local inhibition of the proteasome with MG132 or β-lactone partially blocks FGF22-induced presynaptogenesis. Finally, activation of the proteasome with IU1, a selective inhibitor of Usp14, induces *per se* clustering of synaptic vesicles. Taken together these results show that local activation of the proteasome has a role in presynaptic differentiation. Future work will be carried out to unravel the molecular mechanisms leading to the assembly of the presynapse.

[1] Van Roessel, P. et al. Cell 119, 707–18 (2004)
[2] Liao, E. H. et al. Nature 430, 345–50 (2004)
[3] Nakata, K. et al. Cell 120, 407–20 (2005)
[4] Bingol, B. & Schuman, E. M. Nature 441, 1144–8 (2006)

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P60. Joana REIS PEDRO

Coimbra, Portugal
joan.reispedro@gmail.com

Intra-axonal translation of β–actin is required for presynaptogenesis

Joana R Pedro[1], Maria Joana Pinto[1,2], Rui O Costa[1], Hyun R Ryu[3], Noo Li Jeon[3,4], Samie R Jaffrey[5], Ramiro D Almeida[1]

1. Center For Neuroscience and Cell Biology University of Coimbra Coimbra, Portugal
2. PhD Programme In Experimental Biology And Biomedicine PDBEB
3. WCU Multiscale Mechanical Design Seoul National University Seoul Korea
4. School of Mechanical and Aerospace Engineering Seoul National University

Local translation of axonal mRNAs has been described in recent years, with evidence for local translation in axonal guidance, growth cone collapse and synaptic plasticity. In Aplysia, local protein synthesis has been shown to have relevant role in synapse formation. However, evidences for the requirement of local mRNA translation in presynaptic differentiation are still missing in vertebrate neurons. Here, we show that local protein synthesis is required for FGF22-induced presynaptogenesis. Using chicken ciliary ganglia neurons we observed that FGF22, a presynaptic organizing molecule, induces the differentiation of nerve terminals in fluidic isolated axons as measured by clustering of SV2, a hallmark of synapse formation. Additionally, when protein synthesis is inhibited specifically in axons, SV2 clustering is reduced to basal levels. We also show that a FGF22 stimulus increased F-actin clusters, indicating an increase in β-actin polymerization. Moreover, FGF22 induces intra-axonal translation of a β-actin reporter, a destabilized form of EGFP fused to the 3'UTR of β-actin. Together these results demonstrate that local mRNA translation is required for presynaptogenesis and that β–actin mRNA is a key modulator of this process.

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P61. Natalia H REVELO

Göttingen, Germany
nrevelo@gwdg.de

Inner hair cells functionally separate synaptic vesicle recycling from constitutive membrane trafficking

Natalia H Revelo, Dirk Kamin, Tobias Moser, Silvio O Rizzoli

Dept of Neuro- and Sensory Physiology University Medical Center Göttingen European Neuroscience Institute Göttingen Germany InnerEarLab Department of Otolaryngology University Medical Center Göttingen Germany Collaborative Research Center 889 University of Göttingen, Germany

Most neurotransmitter-releasing cells separate synaptic vesicle recycling from other membrane trafficking processes by isolating the synaptic process in specialized compartments (presynaptic boutons). However, how vesicles are recycled in cells that lack such boutons and use instead release sites directly on the cell soma (somatic active zones) is not known. Would synaptic vesicles recycle through a specialized pathway, or rather use the constitutive endosomal, Golgi or ER-trafficking pathways? Here we selected the auditory inner hair cells (IHCs), as a model to address this question. We visualized membrane trafficking by dye photo-oxidation electron microscopy and by super-resolution optical microscopy of a novel membrane binding probe. We found synaptic vesicle recycling concentrated at the cell base, where the active zones are located. Upon stimulation membrane infoldings appeared in the vicinity of the active zones giving rise to large endosome-like structures that were later processed to synaptic vesicles. This activity-dependent membrane retrieval seems to involve clathrin and dynamin. In resting conditions, we found an abundant constitutive membrane recycling that uses

tubulo-cisternal organelles as endosomal intermediates. Our data suggest that even in the absence of a synaptic bouton, synaptic vesicle recycling is functionally separated from constitutive membrane trafficking. We also show the suitability of our novel probe in the study of endocytosis under high resolution microscopy.

P62. Andreas RITZAU-JOST
Leipzig, Germany
andreas.ritzau-jost@medizin.uni-leipzig.de

KHz transmission at a central synapse

Andreas Ritzau-Jost, Annika Weyhersmüller, Igor Delvendahl, Hartmut Schmidt, Johannes Hirrlinger, Jens Eilers, Stefan Hallermann
European Neuroscience Institute Göttingen, Grisebachstr 5, 37077 Göttingen, Germany
Carl-Ludwig-Institute of Physiology Medical Faculty, University of Leipzig, Liebigstrasse 27, 04103 Leipzig, Germany, Max Planck Institute of Experimental Medicine, Göttingen, Germany

The rate at which signals can be transmitted between single neurons limits the speed of information processing in the central nervous system. Cerebellar mossy fiber boutons display rates of action potentials up to 1 kHz in vivo [1,2]. While rapid reloading of vesicles at the release site likely sustains reliable transmission [3,4], a mechanistic analysis with presynaptic recordings has not been performed. Here, we established paired patch-clamp recordings between cerebellar mossy fiber boutons and granule cells, presynaptic capacitance measurements and deconvolution of postsynaptic currents. We show that presynaptic action potentials are ultrafast (half-width 107 ± 4 µs, n = 44) and efficiently open Ca^{2+} channels to transmit signals at frequencies of at least 1 kHz. We analyze the kinetics and the Ca^{2+}-dependence of transmitter release during trains of action potentials and prolonged depolarization. The findings suggest that heterogeneous vesicle populations with different and partially Ca^{2+}-independent reloading mechanisms contribute to release. Our results show that the heterogeneity among vesicles allows kHz transmission at a central synapse.

[1] Rancz, E.A. et al. High-fidelity transmission of sensory information by single cerebellar mossy fibre boutons. Nature 450, 1245-1248 (2007).
[2] Jörntell, H. & Ekerot, C.F. Properties of somatosensory synaptic integration in cerebellar granule cells in vivo. J. Neurosci. 26, 11786-11797 (2006).
[3] Saviane, C. & Silver, R.A. Fast vesicle reloading and a large pool sustain high bandwidth transmission at a central synapse. Nature 439, 983-7 (2006).
[4] Hallermann, S. et al. Bassoon speeds vesicle reloading at a central excitatory synapse. Neuron 18, 710-723 (2010).

P63. Morgane ROSENDALE
Bordeaux, France
morgane.rosendale@u-bordeaux2.fr

Detecting the formation of endocytic vesicles in the soma and dendrites of live cultured neurons

Morgane Rosendale, Damien Jullié, William Abdou, David Perrais
Interdisciplinary Institute of Neuroscience, CNRS & University Bordeaux Segalen, Bordeaux, France

Endocytosis is a fundamental process regulating the number of receptors at the surface of cells. In neurons, blocking clathrin mediated endocytosis (CME) leads to an increase of the number of postsynaptic AMPA receptors within minutes and blocks long term depression of synaptic transmission. Moreover, clathrin coated pits are concentrated near synapses, consistent with a specific role of CME in synaptic plasticity. However, unlike in most cell lines, clathrin coated structures seem to be very stable in neurons, making it difficult to study endocytosis in those cells in a conventional way. To clarify the link between CME and synaptic plasticity, it would be important to directly detect the formation of clathrin coated vesicles (CCVs) in live neurons. To do so, we have adapted a method based on extracellular pH changes which detects the moment when transferrin receptors labeled with superecliptic phluorin (TfR-SEP) are isolated from these changes, i.e. the moment of CCV formation (Merrifield, Perrais & Zenisek, Cell 2005). We have imaged cultured hippocampal neurons (9-15 days in vitro), transfected with TfR-SEP, with TIRF (total internal reflection fluorescence) microscopy. When neurons

were submitted to this so-called ppH protocol, we detected the formation of CCVs throughout the somato-dendritic compartment at sites of clathrin structures labeled with CLC-DsRed. We controlled that the ppH protocol in itself did not affect CME in neurons: the frequency of detected CCVs was constant during 10 to 15 min recordings, and transferrin labeled with Alexa568 was normally internalised and trafficked within cells. However, application of low pH solution evokes large currents in these cells, due to the opening of acid sensing ion channels (ASICs). Blocking ASICs with amiloride (500 µM) effectively prevented the induction of these currents without affecting the formation of CCVs. Moreover, when the low pH solution was replaced by a cell impermeable quencher of SEP fluorescence, namely Trypan Purple, we could monitor the formation of CCVs with similar frequencies, demonstrating that the activity of endocytic pits can be recorded in live neurons in basal conditions. We will therefore now use this protocol to monitor possible changes in the rate of endocytosis during protocols inducing synaptic plasticity.

P64. Laura RUSCHKIES
Hamburg, Germany
laura.ruschkies@zmnh.uni-hamburg.de

Conditional knockout and characterization of microtubule severing enzymes in mice

Laura Ruschkies, Irm Hermans-Borgmeyer, Susanne Fehr, Matthias Kneussel
Institute for Molecular Neurogenetics, Center for Molecular Neurobiology, Universitätsklinikum Hamburg-Eppendorf, 20251 Hamburg, Germany

Microtubules serve as molecular tracks along which motor proteins transport a variety of cargoes to appropriate subcellular destinations. The microtubule track can be modified in an activity-dependent manner by various posttranslational modifications (PTMs). Recent findings indicate that dynamic microtubules can enter dendritic spines thus implying a regulatory role in synaptic function. In addition to dynamic instability, severing breaks the microtubule polymer into shorter pieces. In the “cut and run” model it is proposed, that the shorter fragments can be mobilized by motor proteins or by “treadmilling” leading to a reorganization of the microtubule cytoskeleton. The AAA-ATPases spastin, katanin and fidgetin are the most common microtubule severing enzymes the mutation of which are associated with neurological disorders. To investigate the role of altered microtubule dynamics in adult neuronal plasticity we disrupted the function of spastin and katanin in postnatal mouse forebrain.

P65. Mariano SOIZA-REILLY
Paris, France
mariano.soiza-reilly@inserm.fr

Developmental synaptic innervation of the mouse dorsal raphe nucleus using array tomography

Mariano Soiza-Reilly, Patricia Gaspar
Institut du Fer à moulin, INSERM UMR-S 839

Neurons located in the dorsal raphe nucleus (DR) represent the main source of forebrain serotonin, regulating stress and emotional states. Dysfunction of DR networks is involved in the pathophysiology of affective disorders including anxiety and depression. Moreover, maladaptive formation/refinement of neural circuits within the DR could contribute to vulnerability to affective disorders later in life. However, the synaptic organization of the developing DR remains unknown. In this study, we examined synaptic innervations of the developing mouse DR using the novel and quantitative high-resolution immunofluorescence technique, array tomography (AT). AT is a light microscopy-based method involving immunolabeling and imaging of ultrathin (70 nm) serial sections. Sections may be subjected to multiple rounds of immunolabeling, and resulting images can be rendered in 3D. This allows to map and quantitatively analyze multiple antigens as well as their relationships to each other in the same tissue volume. We focused on glutamate and GABA synaptic innervations because they represent the main synaptic inputs to the nucleus. Glutamate axons arising from cortical and subcortical structures were detected by immunolabeling for the vesicular glutamate transporters, VGLUT1 and VGLUT2 respectively. GABA axons were labeled against the GABA synthetic enzyme GAD2. Specific presynaptic markers were combined with general markers for synaptic boutons (e.g. Synapsin 1) as well as with postsynaptic markers (e.g. PSD-95). Additionally, serotonin cells were identified by the presence of the enzyme tryptophan hydroxylase. The results showed that at early postnatal ages both excitatory glutamate and inhibitory GABA

synaptic innervations are abundantly present in the DR. Additionally, they frequently established direct associations with serotonin neurons. These observations indicate that AT represent a unique approach to quantitatively examine the spatial organization of multiple populations of synapses in the same volume of tissue. This approach will increase our understanding of how the DR circuitry develops, and if possible alterations in the synaptic connectivity could contribute to maladaptive mechanisms underlying affective disorders.

P66. Jennifer STANIC
Montreuil, France
jennifer.stanic@unimi.it

Rabphilin 3A interacts with GluN2A NMDA receptor subunit in the post-synaptic density

Stanic J, Caldarelli A, Canonico PL, Genazzani AA, Gardoni F, and Di Luca M
Department of Pharmacological and Biomolecular Sciences University of Milano Via Balzaretti 9 20133 Milano Italy, DISCAFF & DFB Center Università degli Studi del Piemonte Orientale "A. Avogadro" Novara Italy

NMDA receptor subunit composition strictly commands receptor function and pharmacological responses. The identity of the GluN2 subunit regulates biophysical and pharmacological properties of the receptor and influences receptor assembly, signalling and trafficking to the postsynaptic membrane. Recently, a two-hybrid screening to find potential proteins interacting with the C-terminal tail of the GluN2A subunit of the NMDA receptor has highlighted Rabphilin 3A (Rph3A) as a potential partner. Rph3A is a synaptic vesicle-associated protein that was first identified as a binding partner of the GTP-bound Rab3A, a member of the Rab family of GTPases implicated in vesicle docking/fusion reactions. Moreover, different studies have indicated that Rph3A can regulate exo- and endocytosis processes at synaptic sites. Firstly, we verified its interaction with GluN2A by immunoprecipitation and GST pull-down experiments. Subcellular fractionation assays revealed that Rph3A is present in Triton Insoluble Postsynaptic compartment and post-synaptic density (PSD) fractions. Immunofluorescence studies performed in neuronal hippocampal cultures, confirmed biochemical experiments revealing Rph3A colocalization with PSD-95 (marker of the postsynaptic compartment) and GluN2A. These results strongly indicate the presence of Rph3A in the PSD compartment and suggest a function of Rph3A in the modulation of GluN2A localization.

P67. Erica TAGLIATTI
Genova, Italy
erica.tagliatti@iit.it

Phosphorylation of synapsin i by Cdk5 sets the ratio between the resting and recycling pools of synaptic vesicles at hippocampal synapses

Erica Tagliatti, Anne M. G. Verstegen, Gabriele Lignani, Antonella Marte, Anna Corradi , Flavia Valtorta, Daniel Gitler, Franco Onofri, Anna Fassio, Fabio Benfenati
Fondazione Istituto Italiano di Tecnologia

Synapsins (Syns) are synaptic vesicle (SV)-associated phosphoproteins involved in the regulation of neurotransmitter release and their phosphorylation by serine/threonine kinases increases SV availability for exocytosis. SynI is one of the presynaptic substrates of cyclin-dependent kinase-5 (Cdk5) that phosphorylates it in the C-terminal region at Ser549 (site-6) and Ser551 (site-7). Moreover, Cdk5 was recently reported to downscale neurotransmission by sequestering SVs in the release-reluctant resting pool. We have investigated whether the phosphorylation of SynI by Cdk5 is physiologically regulated and impacts on SV dynamics. We have observed that SynI is constitutively phosphorylated by Cdk5 and its phosphorylation increases the binding to F-actin. The effects of Cdk5 inhibition on resting pool size and kinetics of pool depletion is virtually abolished in SynI KO neurons as well as in SynI KO neurons expressing the non-phosphorylatable 6,7A- or 7A-SynI mutants.The expression of either mutant also increased the frequency of miniature postsynaptic currents and the amplitude of evoked release. The observation that the single site mutant 7A-SynI phenocopies the effects of the absence of SynI identifies site-7 as the central switch in mediating the Cdk5 effects and demonstrates that SynI is necessary and sufficient for achieving the effects of the kinase on SV trafficking. Finally, the phosphorylation state of SynI by Cdk5 is regulated during chronic modification of neuronal activity, indicating

that SynI directly contributes to the Cdk5-mediated homeostatic mechanism that fine tunes the recruitment of SVs to the active recycling pool.

P68. Tomi TAIRA
Helsinki, Finland
tomi.taira@helsinki.fi

Synaptic kainate receptors in CA1 interneurons gate the threshold of theta frequency-induced long-term potentiation

Clarke VJR, Lauri SE, Collingridge GLC, Taira T
Neuroscience Center University of Helsinki and University of Bristol

Theta oscillations (4-12 Hz) in neuronal networks are known to predispose the synapses involved to plastic changes and may underlie their association with learning behaviours. The lowered threshold for synaptic plasticity during theta oscillations is thought to be due to decreased GABAergic inhibition. Interneuronal kainate receptors (KARs) regulate GABAergic transmission and are implicated in theta activity; however, the physiological significance of this regulation is unknown. We show that during theta activity there is excitatory postsynaptic drive to CA1 interneurons mediated by KARs. This promotes feedforward inhibition of pyramidal neurons, raising the threshold for induction of theta-burst LTP. These results identify a novel mechanism whereby the activation of postsynaptic KARs in CA1 interneurons gate changes in synaptic efficacy to a physiologically-relevant patterned stimulation.

P69. Agnès THALHAMMER
Genova, Italy
agnes.thalhammer@iit.it

Compartmentalized expression of PQ-type Ca2 channel isoforms shapes neurotransmitter release and short-term synaptic plasticitya central synapses

Agnes Thalhammer[1], Yaroslav S Ermolyuk[3], Thierry Nieus[1], Kirill E Volynski[3], Tuck W Soong[4,5], Yukiko Goda[2,6], Lorenzo A Cingolani[1,2]
1. NBT, IIT, 16163 Genova, Italy
2. MRC LMCB, UCL, London ,UK
3. Institute of Neurology, UCL, London UK
4. Dept of Physiology National University of Singapore, Singapore
5. National Neuroscience Institute, Singapore
6. RIKEN Brain Science Institute, Wako, Saitama, Japan

Cav2.1 (P/Q-type) channels are the predominant voltage-gated calcium channels responsible for initiating synaptic transmission at fast synapses in the central nervous system (CNS). Alternative splicing of Cav α subunits generates a multitude of Ca2+ channel isoforms with distinct biophysical properties, and developmental and regional expression patterns. So far, however, it is not known how these differences in function and localization affect synaptic transmission. Here, we investigate how two major Cav2.1 channel isoforms (Cav2.1[EFa] and Cav2.1[EFb]), which display distinct calcium-dependent facilitation (CDF) in non-neuronal cells, affect neurotransmitter release and short-term synaptic plasticity in hippocampal neurons by recording excitatory synaptic currents, and by imaging presynaptic calcium transients and synaptic vesicle release. We found that the two isoforms affected short-term synaptic plasticity in opposite directions: presynaptic expression of Cav2.1[EFa] led to strong synaptic depression whereas Cav2.1[EFb] to pronounced synaptic facilitation. We provide evidence that this is not due to the differential biophysical prosperities of the two channels but due to their different localization within the presynaptic bouton and differential coupling to the neurotransmitter release machinery. Considering that Cav2.1[EFa/b] isoforms display different developmental and regional expressions, our findings indicate the alternative splicing of Cav2.1 α subunits as a new mechanism contributing to the variability in release probability and short-term synaptic plasticity within the CNS. This work has been been supported by the Medical Research Council, the Italian Institute of Technology and by the ERC.

P70. Cezar TIGARET

Bristol, United Kingdom
Cezar.Tigaret@bristol.ac.uk

Postsynaptic spine Ca2 transients do not correlate with spike timing-dependent plasticity in mature CA1 Hippocampal pyramidal neurons

Cezar M Tigaret, Josef Sadowski, Jack R Mellor
School of Physiology and Pharmacology University of Bristol

Induction of spike timing-dependent synaptic plasticity at glutamatergic Schaffer collateral synapses onto CA1 hippocampal pyramidal neurons requires specific timing between pre- and post-synaptic activity. An attractive hypothesis states that the NMDA receptor-generated transient cytosolic Ca2+ rises at the postsynaptic spines (EPSCaT) determine the direction of plasticity, with large Ca2+ transients leading to potentiation and small Ca2+ transients leading to depression [1,2]. However, this hypothesis has been directly tested experimentally only at immature cortical synapses [3]. Using two-photon Ca2+ fluorescence imaging [4] in acute hippocampal slices from adult rats we recorded the EPSCaTs triggered at apical dendritic spines in stratum radiatum by pairing local presynaptic stimuli with somatically-evoked postsynaptic activity. We then correlated these transients with the ability of the pairing protocols to generate synaptic plasticity. Recordings were performed in whole-cell current clamp, at 36˚C, under GABAA receptor inhibition (50 µM picrotoxin). Pairing single presynaptic stimuli with somatic spikes yielded larger EPSCaTs compared to responses to single presynaptic stimuli alone. This effect was stronger when presynaptic stimulus was paired with two back-propagated action potentials within 10 ms and was independent of the timing sign. In contrast, induction of NMDA receptor-dependent LTP required pairing of at least two back-propagated action potentials 10-50 ms following, but not preceding, a single presynaptic stimulus. Surprisingly, pairs of presynaptic stimuli at 100 Hz generated larger EPSCaTs than the LTP-inducing pairing protocols but failed to induce plasticity indicating that the magnitude of EPSCaT is not sufficient to determine the direction of synaptic plasticity at mature Schaffer collateral synapses. Furthermore, induction of spike timing-dependent LTP required the activation of T- and L-type voltage-sensitive Ca2+ channels and was specifically blocked by the mGlu1 antagonist YM298198 . Thus, our data indicate that mGlu1 activation and distribution of voltage-sensitive Ca2+ fluxes at the spine may constitute factors in addition to NMDA receptors that determine the induction of spike timing-dependent plasticity.

References:

[1] Buchanan, K.A., and Mellor, J.R. (2010) The activity requirements for spike timing-dependent plasticity in the hippocampus. Frontiers in Synaptic Neuroscience, 2, doi: 10.3389/fnsyn.2010.00011
[2] Shouval, H.Z., Bear, M. F., Cooper, L.N. (2002) A unified model of NMDA receptor-dependent bidirectional synaptic plasticity Proc. Natl. Acad. Sci. USA. 99(16): 10831-10836
[3] Nevian, T. and B. Sakmann (2006). "Spine Ca2+ signaling in spike-timing-dependent plasticity." J Neurosci 26(43): 11001-11013
[4] Yasuda, R., E. A. Nimchinsky, et al. (2004). "Imaging calcium concentration dynamics in small neuronal compartments." Sci STKE 2004(219): pl5.

P71. Marilyn TIRARD

Goettingen, Germany
tirard@em.mpg.de

Analysis of neuronal SUMOylation using TAP-SUMO knock-in mice

Marilyn Tirard, Silvia Ripamonti, Trayana Stankova, Nils Brose
Dept of Molecular Neurobiology Max Planck Institute of Experimental Medicine 37075 Goettingen Germany

SUMOylation is a posttranslational modification akin to ubiquitylation, involving the covalent attachment of a SUMO peptide (SUMO1, SUMO2, or SUMO3) to a lysine residue of a target protein. SUMOylation can affect the localization, interactions, function, or stability of substrate proteins, and participates in many cellular signaling pathways that intersect with other posttranslational regulatory processes such as phosphorylation or ubiquitylation. While the role of ubiquitylation and phosphorylation in neuronal function and dysfunction has received a lot of attention in the past, interest in neuronal SUMOylation has developed more recently. SUMO

targets are predominantly found in the cell nucleus, where they often regulate transcription factors. However, a limited number of studies indicate that SUMOylation also occurs outside the nuclear compartment. For example, the kainate receptor GluK2, several voltage-gated ion channels, and proteins of the synaptic scaffold were reported to be SUMOylated, indicating that SUMOylation may regulate neuronal excitability and synaptic transmission. Key problems in this context are (i) that SUMOylated states of proteins are transient in vivo and labile in vitro because of isopeptidases that revert SUMOylation, (ii) that usually only a small fraction of a given substrate protein is SUMOylated, which confounds the discovery and validation of new substrates, and (iii) that reliable antibody tools for the localization and affinity purification of natively SUMOylated proteins are largely lacking. To circumvent these problems, we generated a knock-in (KI) mouse line that expresses double affinity-tagged His6-HA-SUMO1 instead of wild-type (WT) SUMO1 from the SUMO1 locus. Focusing on brain tissue, we found that the KI mouse line represents an excellent mammalian model for the localization and identification of new SUMO1-conjugated proteins. In combination with genetic or pharmacological manipulations, the KI can be used to study SUMOylation in vivo in a wide variety of physiological and pathophysiological contexts. Using these KI mice, we identified Zbtb20 as a novel SUMO1 substrate. Zbtb20 is a transcription factor that regulates the expression of genes involved in the development of the hippocampus, a brain structure with a major role in learning and memory. We further found that primary hippocampal neurons lacking Zbtb20 have a reduced complexity of their dendritic arbor. We are currently examining the role of Zbtb20 SUMOylation in neuronal differentiation by employing a KO-rescue approach.

P72. Jan TØNNESEN
Bordeaux, France
jan.tonnesen@inserm.fr

Nanoscale spine neck morphology regulates compartmentalization of synapses

Tønnesen J, Nägerl UV.
Institut interdisciplinaire de neurosciences, Université Bordeaux, CNRS UMR 5297, F-33000 Bordeaux, France

Dendritic spines are the postsynaptic component of excitatory synapses, representing basic computational units of the brain. They are physically segregated structures that are well-suited for compartmentalized biochemical signaling, which is thought to be critical for the function and regulation of synapses. However, the basis of synapse compartmentalization is currently unknown, as live spine morphologies have not been well characterized due to technical limitations in microscopy, which have impeded visualization of important morphological details like the spine neck. It is therefore unknown to what extent synapse compartmentalization is dictated by morphology or by intracellular factors. We combine superresolution (stimulated emission depletion) STED microscopy, offering around 50 nm optical resolution, together with fluorescence recovery after photo-bleaching (FRAP) measurements of freely diffusible cytosolic molecules to determine the structural aspects of synapse regulation in live spines of CA1 neurons in mouse organotypic slices. Our images reveal a broad continuum of spine shapes and sizes, defying commonly applied classification schemes. They further disclose that spines with short necks may erroneously appear stubby when imaged by two-photon microscopy and that true stubby spines are in reality exceedingly rare. By investigating diffusion of fluorophores with sizes corresponding to essential intracellular messengers, we show that spine neck width is the key structural determinant of synapse compartmentalization, which influences molecular diffusion in a highly non-linear and dynamic way. In addition, our data allow us to estimate the electrical neck resistance in three independent ways, yielding remarkably similar results, covering ranges from few MOhm to around one GOhm. Interestingly, our data suggest that biochemical and electrical compartmentalization are differentially regulated parameters for a substantial fraction of synapses, revealing a new layer of complexity for the structure-function relationship of synapses.

P73. Philipp TREPTE
Berlin, Germany
philipp.trepte@mdc-berlin.de

Generation of an interactome map for synaptic proteins

Philipp Trepte, Angeli Möller, Martin Schäfer, Oksana Sorokina, Miguel Andrade, Douglas Armstrong, Erich Wanker
Max-Delbrück-Center for Molecular Medicine, The University of Edinburgh

Synapses are highly structured compartments that enable information transfer between neurons. Recent proteomics studies have increased our knowledge on the protein composition of synapses. However, it remains mostly unclear how synaptic proteins function and assemble into multiprotein complexes to perform their specific tasks. Also, it is still largely unknown how dysfunctions in synaptic proteins contribute to severe neurological disorders like schizophrenia, epilepsy or Alzheimer's disease. Thus, the systematic analysis of synaptic protein-protein interactions (PPI) might provide new insights in key synaptic processes as well as shed light on important disease mechanisms. Here, we utilized different yeast-two hybrid (Y2H) screening methods and integrated previously published interactions to create a comprehensive PPI network connecting 1,340 synaptic proteins via 16,722 PPIs. Importantly, we observed that known pre- and postsynaptic proteins separate in this PPI network, emphasizing the validity of our network biology approach. Moreover, using a computational strategy we predicted novel proteins to be involved in key synaptic processes such as exo- and endocytosis. Finally, proteins that associate with known disease proteins such as the amyloid precursor protein (APP) were defined and experimentally confirmed with independent PPI detection methods. Thus, we suggest that our interactome network is a valuable resource for detailed experimental investigations that will increase our understanding of important synaptic processes as well as disease mechanisms underlying synaptopathies.

P74. Haijun TU
Villeurbanne, France
haijtu@gmail.com

A novel PUNCTINMADD-4 pathway required for GABA receptor clustering in Caenorhabditis elegans

Haijun Tu, Bérangère Pinan, Marie Pierron, Tingting Ji, and Jean-Louis Bessereau
Université Claude Bernard Lyon 1

GABAA receptors are prominent ligand-gated chloride channels that mediate fast inhibitory neurotransmission in the mammalian central nervous system. In C. elegans, only one locus, unc-49, encode three GABAA receptor subunits, which comprise UNC-49A, UNC-49B, and UNC-49C isoforms. UNC-49/GABA receptors localize at neuromuscular junctions where they cluster in front of GABA release sites. Up to date, the mechanisms regulating the postsynaptic organization of inhibitory neuromuscular junctions remain unknown in C. elegans. To identify the molecules required to form UNC-49 clusters, we tagged the UNC-49B subunit with the tagRFP and used the MosSCI technique to generate a single-copy transgene driving UNC-49B-tagRFP expression under the control of its own promoter. Tagged GABARs are functional and detected as bright synaptic puncta. We mutagenized this strain with EMS and performed a visual screen for abnormal GABARs distribution. From about 2,000 mutagenized haploid genomes, we isolated 13 mutants which were outcrossed and sequenced. Among these mutants, we identified mutations madd-4/punctin that cause a dramatic reduction of GABARs at neuromuscular junctions (NMJs). Madd-4/punctin mutation disrupts synaptic receptors clustering at both cholinergic and GABAergic NMJs (B. Pinan, H. Tu, et al., unpublished data). In addition, we identified mutations that specifically affect synaptic expression of GABARs. Results in progress will be presented at the meeting.

P75. Emilia TURCO
Torino, Italy
emilia.turco@unito.it

p140Cap regulates memory and synaptic plasticity through Src- and CitN-mediated RhoA control

D. Repetto[1], P. Camera[1], R. Melani[2,3], N. Morello[4], E. Calcagno[4], R. Tomasoni[5], F. Bianchi[1], G. Berto[1], I. Russo[1], M. Giustetto[4], N. Berardi[2,3], T. Pizzorusso[2,3], M. Matteoli[5], P. Di Stefano[1], E. Turco[1], F. Di Cunto[1], P. Defilippi[1]
1. MBC University Of Torino
2. CNR Pisa
3. University of Florence, Firenze
4. Dept of Neuroscience, Torino
5. Dept of Biotechnology University of Milan

A major challenge in the neuroscience field is the identification of molecules and pathways that control synaptic plasticity and memory. In particular dendritic spines (DS) play a pivotal role in these processes, as the

major sites of excitatory synapses in neuronal communication. Previous studies have shown that the scaffold protein p140Cap localizes into dendritic spines and that its knockdown negatively modulates spine shape in culture. However, so far, there is no information on its in vivo relevance. By using a knock-out mouse model, we here demonstrate that p140Cap is a key element for both learning and synaptic plasticity. Indeed, p140Cap -/- mice are impaired in object recognition test, as well as in LTP and in LTD measurements. The in vivo effects of p140Cap loss are presumably attenuated by non-cell autonomous events, since primary neurons obtained from p140Cap -/- mice show a strong reduction of mushroom spines number and abnormal organization of synapse-associated F-actin. These phenotypes are most likely caused by a local reduction of the RhoA to Cofilin signalling cascade, which can be controlled by p140Cap through its capability to directly inhibit the activation of Src kinase and by its binding to the scaffold protein citron Cit-N. Altogether, our results provide new insight into how protein associated to dynamic microtubules may regulate spine actin organization through interaction with post-synaptic density components.

P76. Silvia VIANA DA SILVA

Bordeaux, France
silviamvsilva@gmail.com

Synapse specific alterations in morphology and function of CA3 pyramidal cells in a mouse model of Alzheimers disease

Viana da Silva S[1,2,3], Labrousse V[2,3], Haberl M[4], Gorlewicz A[2,3], Blanchet C[2,3], Frick A[4], Mulle C[2,3]
1. PDBEB CNC, University of Coimbra, Portugal
2. Univ. Bordeaux, IINS, UMR 5297, F-33000 Bordeaux, France
3. CNRS, IINS, UMR 5297, F-33000 Bordeaux, France
4. INSERM U862, F-33000 Bordeaux, France

Synapse loss is known to correlate with the progression of the Alzheimer's disease. Amyloid-beta (AB) peptide, generated in neurons by a series of amyloid precursor protein (APP) cleavages is involved in alterations of synaptic transmission prior to neuronal degeneration, amyloid plaque deposition and neurofibrilary tangles formation. Early disturbance in synaptic processes involved in learning and memory have been reported in several transgenic mouse models. We evaluated synaptic function during the course of the development of the disease in double transgenic APP/PS1 mice, which display accelerated cognitive deficits prior to amyloid plaques formation. We focused on hippocampal CA3 region, which has not yet been examined, although it is a key structure for memory encoding, and is largely affected in AD. CA3 pyramidal cells receive different types of glutamatergic inputs, from dentate granule cells (mossy fibers), from other CA3 pyramidal cells (associative/commissural fibers), and from the entorhinal cortex (perforant path synapses). These inputs differ in terms of position along the dendrites, spine structure, glutamate receptor equipment, presynaptic properties, and types of plasticity expressed (short and long term-synaptic plasticity). We thus compared how chronic AB peptide deposition in APP/PS1 mice differentially affected these parameters by combining whole cell patch-clamp recordings in acute hippocampal slices with confocal microscopy of virally infected cells to study morphological changes. We found that mossy fiber synapses (Mf) are relatively spared in the diseased CA3 as compared to associative-comisssural (A/C) synapses both in terms of electrophysiological properties and in terms of morphology. We found a striking loss of NMDA-dependent LTP at A/C synapses which could be correlated with a decrease in the volume of spines, but not in the relative amount or properties of synaptic NMDA receptors. These result highlight the fact that chronic AB deposition does not affect all glutamatergic synaptic contacts in the same manner, and provide clues about the molecular mechanisms underlying plasticity impairment prior to amyloid plaque deposition.

P77. John WESSELING

Pamplona, Spain
jwesseling@unav.es

Parallel recruitment of slow and fast releasing vesicles to the RRP at Calyx of held synapses

Kashif Mahfooz, Robert Renden, and John Wesselnig
Univ. de Navarra, Univ. of Nevada at Reno, Univ de Navarra, Spain

Although built of similar molecules, gigantic Calyx of Held synapses are used two orders of magnitude more heavily than standard excitatory synapses. Presynaptic vesicle trafficking seems to operate more quickly, but precise estimates at intact synapses factoring out differences in size that can be directly compared to standard synapses are not available. We have now conducted such experiments in slices of 14 - 19 day old mice and rats. Vesicles were recruited to the readily-releasable pool (RRP) with a rate constant of ~ 3.9/s during maximal use at room temperature, which is in-line with estimates extrapolated from previous experiments at both intact and patch clamped calyces, and ~ 15-fold faster than the maximum at excitatory hippocampal synapses. Submaximal use depleted the RRP of fast-releasing vesicles, but left the slow-releasing subcomponent in a partially full standing steady state. Surprisingly, once released, the rate of replacement for slowly releasing vesicles was similar to the rate for fast releasing vesicles, contrary to the calyx-specific concept that slowly releasing vesicles are always recruited more rapidlly. Further analysis suggested that slowly and fast releasing vesicles are recruited and released in parallel and argued against the alternative concept that slowly releasing vesicles constitute an upstream pool.

P78. Stefania ZAMBETTI
Milan, Italy
stefania.zambetti@gmail.com

The actin capping activity of Eps8 is required for spine morphogenesis and plasticity

Stefania Zambetti[1,3], Raffaella Morini[2,3], Andrea Donzelli[3], Andrea Disanza[4], Lucrezia Folladori[3], Daniela Braida[3], Chiara Nicolini[5], Giuliana Fossati[2,3], Maria Cristina Regondi[6], Carolina Frassoni[6], Giorgio Scita[4], Mariaelvina Sala[1,3], Margaret Fahnestock[5], Michela Matteoli[2,3], and Elisabetta Me

1. CNR Institute of Neuroscience, Milan, Italy
2. Humanitas Clinical and Research Center, Milan, Italy
3. Dep of BIOMETR, Milan, Italy
4. IFOM Foundation, Milan, Italy
5. Dep of Psychiatry And Behavioural Neurosciences Canada
6. Istituto Neurologico Carlo Besta, Milan, Italy

Actin-based remodeling underlies spine structural changes occurring during synaptic plasticity, the process that constantly reshapes the circuitry of the adult brain in response to external stimuli, leading to learning and memory formation. A positive correlation exists between spine shape and synaptic strength and, consistently, abnormalities in spine number and morphology have been described in a variety of neurological and neuropsychiatric disorders. Dissecting the molecular mechanisms involved in synapse formation and plasticity could pave the way for the rational design of new therapeutic approaches for the treatment of intellectual disability. In the present study, we demonstrate that the actin-regulating protein, Eps8, is recruited to the spine head during chemically induced long-term potentiation in culture and that spine morphogenesis and plasticity are impaired in hippocampal neurons established from E18 Eps8 knockout mice. Furthermore, we show that the actin-capping activity of the protein is required for spine structural remodeling upon LTP induction. Accordingly, mice lacking Eps8 display immature spines, which are unable to undergo potentiation, and are impaired in cognitive functions and sociability. Additionally, we found that reduction in the levels of Eps8 occurs in brains of patients affected by autism compared to controls. Our data reveal the key role of Eps8 actin-capping activity in spine morphogenesis and plasticity and indicate that reductions in actin-capping proteins may characterize forms of intellectual disabilities associated with spine defects.

P79. Jonathan ZAPATA
Milan, Italy
zapata.jonath@gmail.com

The X-LID protein SHROOM4 regulates $GABA_B$ receptors trafficking through its association with the microtubule-dependent molecular motor dynein

Zapata J[1], Moretto E[2], Murru L[1], Benedetti L[7], Hannan S[3], Fossati M[1], Heise C[1], Bassani S[1], Valnegri P[1,2], Gardoni F[4], Francolini M[7], Kalscheuer V[5], Bettler B[6] and Passafaro M[1,2]

1. CNR institute of Neuroscience, Department BIOMETRA, University of Milan
2. Dulbecco Telethon Institute.

3. Department of Neuroscience, Physiology, and Pharmacology, University College London.
4. Department of Pharmacological Sciences, University of Milan
5. Max Planck Institute for Molecular Genetics, Department Human Molecular Genetics, Berlin
6. Departement of Biomedicine University of Basel
7. Fondazione Filarete, Milan

Mutations in the SHROOM4 gene cause intellectual disabilities (ID) and its expression and role in the brain was still unknown. Our data reveal that SHROOM4 is present in both cortical and hippocampal primary cultured neurons and localizes at the pre- and post-synapse. siRNA mediated SHROOM4 knock-down leads to a dramatic reduction in spine number, length and width. Furthermore, we detected a significant decrease in the expression level of synaptic markers compared to a siRNA scrambled control. Using different approaches, we showed that the N-terminal PDZ domain of SHROOM4 interacts directly with the C-terminal tail of $GABA_B$ receptor subunit 1 ($GABA_BR1$) (both R1A and R1B). According with the previously demonstrated role of the C-terminal tail of $GABA_BR1$ in its own trafficking, we observed for the first time, that SHROOM4 regulates $GABA_BR1$ intracellular trafficking by modulating its association with the microtubules-dependent molecular motor, dynein. These results support the hypothesis of a trimeric complex, necessary for a correct transport of $GABA_BR1$ receptors into dendrites and for its physiological role. These observations suggest that the symptoms detected in SHROOM4 deficient human patients could arise from reduced level of functional $GABA_B$ receptors.

P80. Benoît ZUBER

Bern, Switzerland
zuber@ana.unibe.ch

Structure and superorganization of acetylcholine receptor-rapsyn complexes

Benoît Zuber, Nigel Unwin
Laboratory of Experimental Morphology, Institute of Anatomy, University of Bern, Switzerland

The scaffolding protein at the neuromuscular junction rapsyn enables clustering of nicotinic acetylcholine receptors in high concentration and is critical for muscle function. Patients with insufficient receptor clustering suffer from muscle weakness However the detailed organization of the receptor-rapsyn network is poorly understood it is unclear whether rapsyn first forms a wide meshwork to which receptors can subsequently dock or whether it only forms short bridges linking receptors together to make a large cluster. Furthermore, the number of rapsyn-binding sites per receptor (a heteropentamer) has been controversial. Here, we show by cryoelectron tomography and subtomogram averaging of Torpedo postsynaptic membrane that receptors are connected by up to three rapsyn bridges the minimum number required to form a 2D network. Half of the receptors belong to rapsyn-connected groups comprising between two and fourteen receptors. Our results provide a structural basis for explaining the stability and low diffusion of receptors within clusters.

P81. Stefano ZUCCA

Bordeaux, France
stefanozucca@hotmail.com

Analysis of synaptic plasticity in CA3 pyramidal cells in vivo using optogenetics tools

Zucca Stefano,Griguoli Marilena,Mulle Christophe
Interdisciplinary Institute for Neuroscience, University of Bordeaux, and CNRS UMR 5297, F-33000 Bordeaux, France

In the hippocampus, signal transmission between the dentate gyrus and CA3 region is mediated by mossy fibers (MF), axons of granule cells (GCs). Very little is known on how granule cells modulate the activity of CA3 neurons in the intact network of a mammalian brain. To address this question we used an optogenetic approach, to selectively control the activity of granule cells, combined with in vivo recordings from CA3 pyramidal neurons, in anesthetized mice. Double-floxed inverted open reading frame (DIO) viral vectors were used to target the expression of ChR2-EYFP to the granule cells layer of the dentate gyrus in POMC-Cre mice. To test the functionality of ChR2 we first performed whole cell recordings from acute hippocampal slices

containing EYFP positive granule cells. Brief pulses of blue light (0.5-1 ms; 470 nm) reliably induced action potentials at different frequencies of stimulation, suggesting that this population of GCs can be optically controlled. We next performed extracellular recordings from dentate gyrus and CA3 area in the anesthetized mouse, to characterize the network activity under basal conditions. The presence of typical hippocampal patterns of activity (i.e. dentate spikes, gamma oscillations) was then used as a criteria for the identification of GCs and CA3 neurons, to obtain whole-cell patch clamp recordings. Finally to test the functional properties of MF-CA3 synapses, we implanted an optical fiber in the dentate gyrus to deliver optical stimulation (1-5 ms, 470nm) while field excitatory postsynaptic potentials (fEPSPs) were recorded in the CA3 region in vivo. Gradual increase of frequency stimulation from 0.05 to 10 Hz, reliably produced facilitation of fEPSPs and confirm that frequency facilitation processes occur in vivo at MF-CA3 similarly to what previously reported in in vitro studies. Further studies at the single cell level will allow us to characterize better the properties of synaptic transmission and plasticity at mossy fibers-CA3 synapses, and gain new insights on this microcircuit in the intact brain.