“Current Trends in Biomedicine”

Workshop:

SYNAPSE FORMATION, SPECIFICATION AND ELIMINATION: FROM MOLECULES TO CIRCUITS

September 25th-27th, 2017
Baeza, Spain

Universidad Internacional de Andalucía
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Organizers: Rafael Fernández-Chacón
Thomas C. Südhof

Sponsored by:

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Centro Investigación Biomédica en Red
Enfermedades Neurodegenerativas

Workshops "Current Trends in Biomedicine" 2017

"Synapse formation, specification and elimination: from molecules to circuits"

Baeza, September 24th-27th, 2017

Organized by

Rafael Fernández-Chacón and Thomas C. Südhof

Sponsor

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SCIENTIFIC PROGRAM

Sunday, September 24th

20:30-21:30h **Get together dinner** at the cafeteria of the "Sede Antonio Machado"

21:30-23:00h **Round Table on Synapse Formation,Specification and Elimination: Key Questions**
(Chairs: **Thomas C. Südhof** and **Rafael Fernández-Chacón)**

Monday, September 25th

Dynamics of synaptic structure and circuits
*(Chair: **Thomas C. Südhof**)*

9:00-9:35 **Thomas Biederer (Tufts University, USA)**
Topography and dynamics of the synaptic cleft

9:35-10:10 **Scott Soderling (Duke University, USA)**
Molecular Analysis of Postsynaptic Inhibition

10:10-10:45 **Nils Brose (Max-Planck-Institute, Germany)**
Formation and Maintenance of Functional Spines in the Absence of Presynaptic Glutamate Release

10:45-11:15 **COFFEE BREAK**

11:15-11:50 **Rafael Fernández-Chacón (IBiS,HUVR/CSIC/Univ. of Seville & CIBERNED, Spain)**
Maintenance of inhibitory presynaptic terminals: mechanisms and consequences upon its failure

11:50-12:10 **Yi E. Sun (UCLA, USA and Tongji Univ. China)**
Using single cell transcriptome analyses to study neural development

12:10-12:30 **Özgun Gökce (Ludwig-Maximilians-Univ., Germany)**
Beyond the D1/D2 receptor dichotomy in basal ganglia circuits

12:30-12:45 **Pablo García-Junco-Clemente (UCLA, USA and IBiS,HUVR/CSIC/Univ. Seville & CIBERNED, Spain)**
Functional connectivity in frontal cortex revealed by in vivo large-scale network imaging

13:00-13:30 **OFFICIAL INAUGURATION OF THE 2017 WORKSHOP SERIES**

13:30-16:00 **LUNCH AND POSTER SESSION**

Circuit assembly and remodeling
*(Chair: **Lawrence Zipursky**)*

16:00-16:35 **Oliver Hobert (Columbia Univ. & HHMI, USA)**
Shaping the sexually dimorphic connectome of the nematode C. elegans

16:35-17:10 **Yishi Jin (Univ. California San Diego, USA)**
Neuronal circuit remodeling in C. elegans

17:10-17:25 **Ben Mulcahy (Mount Sinai Hospital, Canada)**
The ultrastructural sequence of events during developmental remodelling of the C. elegans motor circuit

17:25-17:55 **COFFEE BREAK**

17:55-18:30 **Liqun Luo (HHMI & Stanford University, USA)**
Genetic Dissection of Neural Circuit Assembly

18:30-18:50 **Artur Llobet (Univ. of Barcelona & IDIBELL, Spain)**
Tight temporal coupling between synaptic rewiring of olfactory glomeruli and the emergence of odour-guided behaviour in Xenopus tadpoles

18:50-19:10 **Frank Schmitz (Saarland University, Germany)**
A CASPR1/contactin1-containing cellular adhesion complex at retinal ribbon synapses is an early target in experimental autoimmune encephalitis (EAE), a mouse model of multiple sclerosis

19:10-19:25 **Elizabeth Zúñiga-Sánchez (UCLA & HHMI, USA)**
Dissecting Mouse Retina Development with RNA-seq and CRISPR

19:25-19:40 **M. Neset Özel (UT Southwestern, USA & Free Univ. Berlin, Germany)**
Synaptic Capture: Synapse formation stabilizes filopodial dynamics in Drosophila brain development

19:40-19:55 **Julio Franco (University of Seville, Spain)**
Role of SMN in Synapse Maturation

20:30 **DINNER**

Tuesday, September 26th

Trans-synaptic signaling in circuit sculpting (1)
*(Chair: **Nils Brose**)*

9:00-9:35 **Thomas C. Südhof (HHMI & Stanford Univ., USA)**
The Molecular Logic of Neural Circuits: Role of Cell-Adhesion Molecules Such as Neurexins and Latrophilins

9:35-10:10 **Eunjoon Kim (KAIST, Korea)**
Netrin-G ligands (NGLs) in the regulation of synapse function and specific behaviors

10:10-10:45 **Jean-Louis Bessereau (Univ. of Lyon, France)**
Bridging extra- and intracellular synaptic scaffolds

10:45-11:05 **Markus Missler (Westfälische Wilhelms-Univ., Germany)**
Calcium channel auxiliary subunits team up with α-neurexins to regulate Ca^{2+} influx and release

11:05-11:20 **Elizabeth C. Davenport (UCL, UK)**
An essential role for the tetraspanin LHFPL4 in the cell type-specific targeting and clustering of synaptic GABAA receptors

11:20-13:30 **GUIDED TOUR OF BAEZA**

13:30-16:00 **LUNCH AND POSTER SESSION**

Trans-synaptic signaling in circuit sculpting (2)
*(Chair: **Yishi Jin**)*

16:00-16:35 **Lawrence Zipursky (UCLA & HHMI, USA)**
Dpr and DIP Ligand/Receptor Pairs Regulate Circuit Development in the Drosophila Visual System

16:35-17:10 **P. Robin Hiesinger (Free Univ. Berlin, Germany)**
Simple Rules in Neural Circuit Assembly

17:10-17:45 **Cheng Zhang (Peking University, China)**
The identification of Protein tyrosine phosphatase receptor type O (PTPRO) as a synaptic adhesion molecule that promotes synapse formation

17:45-18:15 **COFFEE BREAK**

18:15-18:50 **Alex Kolodkin (HHMI & Johns Hopkins Univ., USA)**
Neuropilin-2/PlexinA3 Receptors Associate with GluA1 and Mediate Sema3F-dependent Homeostatic Scaling in Cortical Neurons

19:00-19:15 **Sergio Gascón (Ludwig-Maximilians-Univ., Germany & Complutense Univ., Spain)**
Neuronal LRP4 Regulates Synapse Formation in the Developing CNS

19:15-19:30 **Richard Sando (HHMI & Stanford Univ., USA)**
Signaling via the adhesion GPCR Latrophilins regulates excitatory synapse formation and specificity in the hippocampus

19:30-19:45 **Fredrik H. Sterky (University of Gothenburg, Sweden)**
Post-translational Regulation of Neurexins by Carbonic Anhydrase Related Protein CA10

19:45-20:00 **Àlex Bayés (Autonomous Univ. Barcelona, Spain)**
Postsynaptic proteome of the hippocampal trisynaptic circuit

20:00-20:15 **Daniel Enterría-Morales (IBiS,HUVR/CSIC/Univ. of Seville & CIBERNED, Spain)**
Insights into the striatal parvalbumin neurons: towards a specific stimulation of GDNF to protect the nigrostriatal dopaminergic neurons

20:30 **DINNER**

Wednesday, September 27th

Molecular diversity, synapse specification and mental disorders
*(Chair: **Rafael Fernández-Chacón**)*

9:00-9:35 **Peter Scheiffele (Biozentrum, Switzerland)**
Alternative splicing programs for synapse specification and neuronal plasticity

9:35-10:10 **Davide Comoletti (Rutgers University, USA)**
Structural and functional insights into neuronal connectivity

10:10-10:30 **Francisco G. Scholl (IBiS, HUVR/CSIC/Univ. of Seville, Spain)**
Second genetic hits in synaptic adhesion proteins in autism

10:30-10:50 **Takuma Mori (Shinshu University, Japan)**
Down-regulation of Calcium/calmodulin-dependent serine protein kinase (CASK) disrupts excitatory-inhibitory balance of synapses by down-regulation of GluN2B.

10:50-11:20 **COFFEE BREAK**

11:20-11:35 **Mathieu Letellier (Univ. Bordeaux & CNRS, France)**
A unique tyrosine residue in the intracellular domain of neuroligin-1 regulates excitatory versus inhibitory synapse differentiation

11:35-11:50 **Raquel Sánchez-Varo (Univ. of Málaga & CIBERNED, Spain)**
Abeta from APP/PS1 Alzheimer mice hippocampus induced synaptic damage in vivo and in vitro

11:50-12:00 **CONCLUDING REMARKS AND FAREWELL**

12:00 **BUS DEPARTURE TO MADRID AIRPORT**


CarmenFM 2017

LECTURES

Topography and dynamics of the synaptic cleft

Thomas Biederer [1], Karen Perez de Arce [1], Konstantina Liouta [2], Ingrid Chamma [2], Vladan Lucic [3], Thomas A. Blanpied [4], Oliver Thoumine [2]

[1] Department of Neuroscience, Tufts University School of Medicine, Boston, Massachusetts, USA
[2] Institut Interdisciplinaire de Neurosciences, CNRS UMR 5297, Université Bordeaux, 146 rue Léo Saignat, 33077 Bordeaux, France
[3] Department of Molecular Structural Biology, Max Planck Institute of Biochemistry, Martinsried, Germany
[4] Department of Physiology, University of Maryland School of Medicine, Baltimore, Maryland, USA

The assembly of exquisitely structured pre- and post-synaptic specializations underlies synaptic transmission. Trans-synaptic adhesion complexes contribute to synapse formation and maturation and can even instruct these processes. Yet, considerable gaps exist in our understanding of the distribution and sites of action of adhesion molecules within the synaptic cleft. We have mapped the organization of the cleft of excitatory synapses at unprecedented resolution using superresolution imaging and EM approaches. This has revealed first, that the synaptic cleft is structurally patterned; second, the cleft is molecularly organized, with different synapse-organizing proteins marking distinct compartments; and third, select synaptic adhesion complexes shape the edge of the cleft. Advances in molecular labeling techniques that are applicable for live super-resolution imaging have in addition begun to reveal that synaptic adhesion proteins can exhibit different nanoscale dynamics. Our recent results support that the proteins that make up the cleft can undergo activity-dependent changes in their mobility and localization. This progress gains insights into the nanodomains that define the topography of the cleft and the dynamics of synaptic adhesion proteins. These properties of the cleft can be critical for the formation of synaptic junctions and the activity-dependent remodeling of mature synapses.

Molecular Analysis of Postsynaptic Inhibition

Akiyoshi Uezu, Tyler A. Bradshaw, Erik J. Soderblom, Scott H. Soderling

The Departments of Cell Biology and Neurobiology, Duke University Medical School, Durham, North Carolina, USA.

Over the past three decades, purification and analysis of protein complexes at the excitatory postsynapse has led to fundamental insights in neurobiology. These insights include how receptor trafficking, synaptic adhesion, cytoskeletal remodeling, and protein phosphorylation contribute to the synaptic plasticity underlying learning and memory. Moreover, genetic perturbations of excitatory postsynaptic proteins are now known to contribute to developmental brain disorders and psychiatric conditions.

In contrast to the well-studied excitatory synapse, biochemical purification and analysis of the inhibitory postsynaptic specialization has remained largely intractable. Fast inhibitory synaptic inputs onto excitatory neurons modify neuronal membrane potentials, spike timing, and the summation of postsynaptic excitatory potentials. Abnormalities of symmetric (inhibitory) synapses at excitatory neurons are linked to multiple developmental brain disorders (DBDs), including autism spectrum disorders (ASD), neonatal hyperekplexia, intellectual disability (ID), and epilepsy. Yet the molecular mechanisms that regulate the inhibitory postsynaptic structure (termed here iPSD) of excitatory neurons are poorly understood as the proteins at this site were, until recently, obscure. We performed a chemico-genetic proteomic study to resolve the molecular composition of the iPSD as it exists *in vivo*. We discovered a rich diversity of proteins enriched at the iPSD, including several novel proteins as well as proteins encoded by genes whose mutations are implicated in developmental brain disorders. These results and the analysis of their function at the iPSD will be discussed.

Formation and Maintenance of Functional Spines in the Absence of Presynaptic Glutamate Release

Nils Brose

Max-Planck-Institute of Experimental Medicine, Department of Molecular Neurobiology, Hermann-Rein-Straße 3, 37075 Göttingen, Germany

Dendritic spines are the major transmitter reception compartments of glutamatergic synapses in most principal neurons of the mammalian brain and play a key role in the function of nerve cell circuits. The formation of functional spine synapses is thought to be critically dependent on presynaptic glutamatergic signaling. By analyzing CA1 pyramidal neurons in mutant hippocampal slice cultures that are essentially devoid of presynaptic transmitter release, we demonstrate that the formation and maintenance of dendrites and functional spines are independent of synaptic glutamate release. Instead, spinogenesis and spine synapse maintenance are likely caused by a combination of cell-autonomous processes that are dictated by genetic programs, and by signaling via guidance cues, trophic factors, and neuronal and synaptic adhesion systems.

Maintenance of inhibitory presynaptic terminals: mechanisms and consequences upon its failure

Rafael Fernández-Chacón

Instituto de Biomedicina de Sevilla (IBiS, HUVR/CSIC/Universidad de Sevilla) & Dpto. de Fisiología Médica y Biofisica & CIBERNED, Sevilla, Spain

The function of neural networks in the brain depends on the interplay between excitatory and inhibitory neurons. Normal network operation relies on the plasticity and good shape of the synaptic organization of neural circuits from birth to senility. Nevertheless, the mechanisms by which synapses and circuits are maintained along the life are not well understood yet. Furthermore, little is known about the specific susceptibility and maintenance requirements of different synaptic types. Cysteine String Protein-α (CSP-α) is a synaptic co-chaperone that prevents activity-dependent degeneration of nerve terminals. Mutations in the human CSP-α gene cause neuronal ceroid lipofuscinosis characterized by progressive dementia and seizures. Interestingly, synapses formed by fast-spiking parvalbumin-positive (PV+) basket cells progressively degenerate in CSP-α KO mice. On the other hand, PV+ basket cells contribute to sculpt hippocampal circuit architecture by regulating the quiescence of neural stem-cell in postnatal neurogenesis. Interestingly, we have found that CSP-α KO mice suffer from a significant deregulation of postnatal hippocampal neurogenesis that, unexpectedly, it is not secondary to presynaptic degeneration of PV+ terminals. Such a finding uncovers a novel and extrasynaptic role for CSP-α relevant for hippocampal circuitry homeostasis. In addition, in order to investigate the role of CSP-α in specific neuronal populations we have generated CSP-α conditional knock-out mice. In particular, we have generated mice that lack CSP-α in PV+ neurons that develop a striking neurological phenotype. In contrast to conventional CSP-α KO mice, the PV-cell specific CSP-α KO mice do not suffer from early lethality. I will discuss in my presentation the interesting perspectives that the analysis of these mice opens to investigate the long term cell-autonomous mechanisms of synaptic maintenance in fast-spiking neurons.

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Shaping the sexually dimorphic connectome of the nematode *C. elegans*

Oliver Hobert

Columbia University, Howard Hughes Medical Institute, New York, USA

Differences between males and females of the same species include differences in morphology, physiology and behavior. Within the brain, males and females display a number of anatomical sexual dimorphisms often in the form of neurons that are present exclusively in one, but not the other sex. Much less is known about how the vast majority of neurons that are present in both sexes may differ in anatomy, synaptic connectivity or function between the sexes and how these sexual differences develop. The comparison of the EM-reconstructed connectome of the hermaphroditic and male nervous systems of the nematode *Caenorhabditis elegans* reveals the existence of sexually dimorphic synaptic connections between sex-shared neurons. I will describe our efforts to visualize these sexually dimorphic connections in live animals and then show how we used these tools to examine how these dimorphisms in synaptic connectivity arise during sexual maturation. I will describe molecular mechanisms that are involved shaping the sexually dimorphic nature of many of the dimorphically connected neurons.

Neuronal circuit remodeling in *C. elegans*

Yishi Jin

Section of Neurobiology, University of California, San Diego, USA

Neural circuits are dynamic, with activity-dependent changes in synapse density and connectivity peaking during different phases of animal development. In *C. elegans*, young larvae form mature motor circuits through a dramatic switch in GABAergic neuron connectivity, by concomitant elimination of existing synapses and formation of new synapses that are maintained throughout adulthood. We previously showed that the initiation of this synapse remodeling is regulated at the transcriptional level. We recently found that the conserved MYRF proteins promote synapse remodeling following ER to nuclear translocation. Moreover, we have defined a mechanism by which an increase in microtubule dynamics during motor circuit rewiring facilitates new synapse formation. Through genetic screens, we have characterized novel mutations that facilitate directional movement of synaptic cargos. Together, Our study delineates temporally distinct signaling pathways that are required for effective neural circuit refinement.

Genetic Dissection of Neural Circuit Assembly

Liqun Luo

HHMI and Department of Biology, Stanford University

My talk will discuss our recent work on the assembly of the fly olfactory circuit and mouse hippocampal network. Emphasis will be placed on molecules that allow pre- and postsynaptic partners to recognize each other.

The Molecular Logic of Neural Circuits: Role of Cell-Adhesion Molecules Such as Neurexins and Latrophilins

Thomas C. Südhof

HHMI & Dept. of Molecular and Cellular Physiology,
Stanford University School of Medicine, Stanford 94305 (tcs1@stanford.edu)

Neural circuits process information by transmitting and computing signals at synapses, and thus critically depend on the number and location of synapses between the neurons that form the circuit and on the properties of these synapses. We hypothesize that the number, location, and properties of synapses are determined by interactions between pre- and postsynaptic cell-surface recognition molecules and/or signaling molecules, and we refer to the rules by which these molecules construct circuits as the molecular logic of neural circuits. Several cell-surface and signaling molecules contributing to the molecular logic of neural circtuis have been characterized, most prominently presynaptic neurexin cell-adhesion molecules and their various ligands. Although neuropsychiatric disorders such as autism and schizophrenia are poorly understood despite high incidence rates, recent progress in human genetics, revolutionized by advances in sequencing technologies, have identified mutations in a large number of genes that predispose to autism and schizophrenia. No common theme unites the affected genes, but a subset of these genes encodes proteins that function at the synapse, including notably the neurexins. We thus further hypothesize that at least a subset of autism and schizophrenia syndromes are produced by specific impairments in the molecular logic of neural circuits, such that the input/output relations in particular circuits are shifted but not blocked, resulting in a skewed information processing capacity of the brain for a selected set of tasks. In support of this hypothesis, we observed that specific autism- and schizophrenia-associated gene mutations in neurexins and their ligands cause selective alterations in a subset of synapses and circuits that induce discrete specific behavioral abnormalities. Although the analysis of the molecular logic of neural circuits and of its impairment in neuropsychiatric disorders is only at the beginning, the conceptual framework that we outlined above might allow a better understanding of how the brain processes information and of how such information processing becomes altered in autism and schizophrenia. In my presentation, I will describe our work on testing the hypotheses inherent in this conceptual framework, focusing on families of synaptic cell-adhesion molecules such as neurexins and latrophilins. Although incomplete, the studies that I will describe have already led to surprising conclusions about how neural circuits are organized, and provide a perspective for future work.

Netrin-G ligands (NGLs) in the regulation of synapse function and specific behaviors

Eunjoon Kim

Center for Synaptic Brain Dysfunctions, Institute for Basic Science (IBS), and Department of Biological Sciences, Korea Advanced Institute of Science and Technology (KAIST), Daejeon 305-701, Korea

Netrin-G ligands (NGLs, also known as LRRC4 proteins) represent a family of leucine-rich repeat-containing adhesion molecules. There are three known members in the family (NGL-1/LRRC4C, NGL-2/LRRC4, and NGL-3/LRRC4B), and all interact with the abundant postsynaptic scaffolding protein PSD-95 through their C-terminal PDZ-binding motifs. The extracellular regions of NGL-1, NGL-2, and NGL-3 interact with presynaptic netrin-G1, netrin-G2, and LAR family receptor protein tyrosine phosphatases (LAR-RPTPs), respectively. Although the in vitro functions of NGLs have been studied to significant extents, studies on in vivo functions of NGLs are largely lagging behind. In this presentation, I will discuss the in vivo functions of NGL-2 and NGL-3 based on the results from KO mice, with focus on NGL-2/3-dependent regulation of synaptic transmission, synaptic plasticity, and specific behaviors, including social interaction and hyperactivity.

Bridging extra- and intracellular synaptic scaffolds

Xin Zhou, Marine Gueydan, Maelle Jospin, Bérangère Pinan-Lucarré and Jean-Louis Bessereau.
Institut NeuroMyoGène (INMG), University of Lyon, France.

The molecular mechanisms involved in the organization and regulation of chemical synapses is a central longstanding question in neuroscience. A prevalent model for synaptic organization relies on synaptic adhesion molecules that engage trans-synaptic interactions in the synaptic cleft and interact with intracellular protein scaffolds to position the synaptic vesicle release machinery in presynaptic boutons and localize neurotransmitter receptors in the postsynaptic membrane. However, numerous secreted proteins are also able to modulate synaptic organization or function. Among them, some proteins behave as 'synaptic scaffolders', *i. e.* proteins that engage interactions with pre- or postsynaptic components within the synaptic cleft and organize the synapse without necessarily triggering a signal transduction cascade.

Using a series of genetic screens for neurotransmitter mislocalization at the *C. elegans* neuromuscular junction, we identified an anterograde synaptic organizer MADD-4/Ce-Punctin. Different isoforms of this extracellular matrix protein are secreted by cholinergic and GABAergic motoneurons, which specifies the identity of excitatory vs inhibitory identity of post-synaptic domains. At cholinergic neuromuscular junctions, acetylcholine receptor clustering and localization mainly depends on an extracellular scaffold assembled in the synaptic cleft. At GABAergic neuromuscular junctions, Ce-Punctin competitively interacts with neurexin to localize neuroligin receptors at the synapse. Neurologin in turn recruits GABA receptor microclusters that are formed upon interaction with intracellular scaffolding proteins.

The *C. elegans* neuromuscular junction therefore provides a genetically tractable system to investigate *in vivo* the composition and properties of diverse synaptic scaffolds.

Dpr and DIP Ligand/Receptor Pairs Regulate Circuit Development in the Drosophila Visual System

Liming Tan*, Qi Xiao*, Shuwa Xu*, Ying Lin, Juyoun Yoo, S. Lawrence Zipursky

University of California, Los Angeles, Dept. of Biological Chemistry, HHMI, Los Angeles, CA.
**, equal contribution*

The establishment of synaptic specificity involves multiple developmental events, including generation of appropriate numbers of pre- and post-synaptic neurons, targeting of axons to designated areas, pairing of appropriate synaptic partners and the assembly of synapses. The cellular recognition mechanisms underlying many of those processes remain poorly understood. Here, I will discuss recent experiments indicating that Dpr and DIP proteins play multiple roles in regulating the assembly of neural circuits in the medulla region of the Drosophila visual system.

Two subfamilies of the immunoglobulin super family proteins, Dprs (21 paralogs) and DIPs (9 paralogs), are expressed in synaptic partners during development (Carrillo et al, 2015; Tan et al., 2015). DIPs and Dprs physically interact with each other in a complex fashion (Ozkan et al, 2013, Carillo et. al., 2015). Previous studies have shown that DIPγ and its binding partner Dpr11 are required for normal visual system development and development at the larval neuromuscular junction (Carillo et al., 2015). Here we describe our progress in uncovering the functions of another DIP protein, DIPα, in the developing fly visual system in a class of amacrine-like cells called Dm neurons.

In CRISPR induced null mutants of DIP-α, multiple neurons, including Dm4, showed reduced cell numbers; this was also seen in double mutants lacking both Dpr6 and Dpr10, the two Dprs that are expressed in synaptic partners of Dm4 and bind to DIP-α. The cell loss in Dm4 was rescued by targeted expression of anti-apoptotic genes p35 or DIAP1, suggesting that Dm4 cell loss is due to apoptosis. Mutant Dm4 cells die after extending axons into the neuropil, but prior to synapse formation. As the Dpr and DIP proteins are mostly localized to the neuropil, it is likely that early interactions between these proteins on developing neurites play a crucial role for determining the correct number of Dm4 neurons and genetic studies suggest this relies on competitive interactions between neurons. DIP-α, as well as its ligands (Dpr6 and Dpr10), are also required for the survival of two other Dm neurons, Dm1 and Dm12. Another ligand/receptor pair, DIPγ and Dpr11, is required for the survival of Dm8 neurons. Thus, these studies support a role for DIP/Dpr interactions in regulating the survival of Dm neurons.

DIP-α appears to play a later role in development also, as uncovered through genetic mosaic studies. Dm1, Dm4 and Dm12 show defects in synaptic terminal organization, including a marked decrease in branch points, frequent mistargeting of axon terminals to a different synaptic layer, and a reduction in synapse number. These studies provide a glimpse of how these two gene families regulate neural circuit assembly. Many other interacting cell surface proteins are expressed on synaptic partners during development. How these and Dpr/DIP proteins orchestra the remarkable specificity of synaptic connectivity between the more than one hundred neuronal cell types in the medulla neuropil remains a fascinating and challenging problem.

Simple Rules in Neural Circuit Assembly

P. Robin Hiesinger

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14195 Berlin, Germany

What type of information is required to robustly grow a neural circuit? We take an algorithmic approach to brain wiring based on live observation of how connections develop in the *Drosophila* visual system: First, we use non-invasive imaging and computational modeling of neural circuit assembly to identify (often surprisingly simple) rules that may suffice to generate complicated wiring diagrams with precision, robustness, and flexibility. Second, we use genetic perturbation to characterize the cells and molecules that execute these rules.

This talk will focus on 'neural superposition', a well-known example for an iterative wiring principle in fly visual systems, where axonal projections from photoreceptors that reside in different unit eyes, but see the same visual axis, 'wire together' in the first optic ganglion. We previously identified an algorithm based on three simple rules, that, iteratively and synchronously executed, could suffice to ensure the sorting of ~4500 photoreceptor axons according to the principle of neural superposition. A computational model made the surprising prediction, that the main target and synaptic partner cells of these photoreceptor axons, L-cells, may not be required as a target during the sorting process. Sparse ablation and live blocking of L-cell dynamics validated the idea of 'targeting without a target', but also revealed a critical earlier role of L-cells in patterning a two-dimensional 'sorting field'. To understand the role of cell interaction molecules known to play important roles in this process (e.g. CadN, Flamingo), we characterized the rules they execute as part of this developmental algorithm using perturbation and live observation of knock-in variants. Our findings support a model in which synaptic specificity in the fly visual map is largely governed by pattern formation rules, rather than classical target recognition.

Neuropilin-2/PlexinA3 Receptors Associate with GluA1 and Mediate Sema3F-dependent Homeostatic Scaling in Cortical Neurons

Qiang Wang[1], Eleftheria Koropouli[1], Shu-Ling Chiu[2], Ingie Hong[2], Sarah Mitchell[1], Teresa P. Easwaran[1], Natalie Hamilton[1], Ahleah S. Gustina[2], David D. Ginty[3], Richard L. Huganir[2], Alex L. Kolodkin[1]

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Homeostatic plasticity maintains neuronal network stability by adjusting synaptic strength in response to global changes in neural activity. Regulation of AMPA-type glutamate receptor (AMPAR) number at synapses is a major mechanism for controlling synaptic strength during homeostatic scaling. Here, we show that the secreted guidance cue semaphorin 3F (Sema3F) and its neuropilin-2 (Npn-2)/plexinA3 (PlexA3) holoreceptor mediate homeostatic plasticity in cortical neurons. Sema3F–Npn-2/PlexA3 signaling is essential for cell-surface AMPAR homeostatic downscaling in response to an increase in neuronal activity, Npn-2 associates with AMPARs, and Sema3F regulates this interaction. Our findings, in combination with our previous work on secreted semaphorin signaling during cortical and hippocampal development, show that Sema3F–Npn-2/PlexA3 signaling controls the development of dendritic morphology, synaptogenesis, and synaptic plasticity in the mammalian CNS.

Alternative splicing programs for synapse specification and neuronal plasticity

Peter Scheiffele

Biozentrum, University of Basel, Klingelbergstrasse 50-70, 4056 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, providing a molecular code for aspects of synaptic specificity and neuronal plasticity. We will discuss recent studies on the role of alternative splicing programs in shaping the molecular diversity in neurons and contributing to the dynamic modification of neuronal gene expression programs during plasticity.

STRUCTURAL AND FUNCTIONAL INSIGHTS INTO NEURONAL CONNECTIVITY

Sinem Ozgul, Moutse Ranaivoson, Sumie Kakehi, Liam Turk, Sventja von Daake, **Davide Comoletti**

Child Health Institute of New Jersey and Department of Neuroscience and Cell Biology, Robert Wood Johnson Medical School, Rutgers University, New Brunswick, NJ 08901, USA

The synaptic cleft contains a dense material composed by hundreds of different proteins, such as receptors, adhesion proteins, etc., that can be defined as the proteome of the synaptic cleft. For the last half century, one of the key questions in neurobiology has been to understand how these proteins provide the specificity and connectivity to build and maintain trillions of synapses. To infer functional properties from molecular composition we need to understand the function and binding partners of these molecules, the so-called molecular interactome of the cleft. The completion of the human genome and the outstanding technical developments in proteomics and laboratory automation are providing the possibility of mapping protein-protein interactions of the surfaceome at an unparalleled speed and scale. These factors should enable scientist to obtain a comprehensive and accurate protein interaction map of the human central nervous system within the next decade. Importantly, cell surface proteins can be targeted and are easily accessible to systemic drug delivery with compounds that do not cross the cell membrane (e.g. monoclonal antibodies).

We have recently implemented and improved an ELISA based assay for the rapid and unbiased identification of cell surface ligand-receptor interactions. Preliminary screening enabled us to identify two novel ligands for CASPR2. CASPR2 is a cell surface glycoprotein expressed in brain and spinal cord that localizes at the juxtaparanodal region of the nodes of Ranvier where it associates with TAG-1 (Contactin 2, CNTN2). While we could not confirm the CASPR2-TAG-1 interaction, we have identified 2 novel ligands for CASPR2: CNTN1 and TICN2. CNTN1 is a cell surface protein expressed by oligodendrocytes and neurons and it is implicated in the development of myelination and oligodendrocyte precursor cells (OPCs). Preliminary data point to the involvement of CASPR2 in the axo-glial recognition process and we propose that CASPR2 is a key molecule that is sufficient to trigger in vitro and in vivo myelination and it may do so through its association with CNTN1. Furthermore, because TICN2 belongs to a family of proteins implicated in myelination, it also has the potential to be important as a positive or negative modulator of myelination.

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POSTERS SELECTED

FOR

SHORT TALKS

Using single cell transcriptome analyses to study neural development

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The mammalian brain contains billions of neurons and trillions of synapses forming various neural circuitries, through which, sense, movement, thought, and emotion arise. Even with such enormous heterogeneity, all brain cells are differentiated from one layer of neuroepithelial stem cells (NSCs). Our current knowledge about NSC is still quite limited, insufficient to fulfill the goal of achieving good understanding of neurodevelopment and to engage endogenous NSCs to repair degenerated or injured central nervous system (CNS). In the past few years we have developed in depth single cell transcriptome analyses method, which enabled us to reveal the existence of dormant NSCs with in the ventricular surface throughout CNS, and ways to activate those cell. Subsequently we developed Patch-seq technology allowing for coupling of single cell transcriptome analyses with electrophysiological recordings. Recently, droplet-based single cell transcriptome analysis has become a scalable, effective and much more affordable approach to reveal critical molecular signatures of NSCs in relate to their very complex and heterogeneous microenvironment. We analyzed data from 1.3 million E18 mouse cortical, hippocampal single cells as well as tens of thousands of cortical cells from E11 to P0 and into adulthood. New insights will be revealed in the poster. This technology could be effectively applied to circuitry mapping to reveal the molecular logic of circuitry functions.

Beyond the D1/D2 receptor dichotomy in basal ganglia circuits

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The striatum is the focal point of a large number of brain disorders. The major neuronal cell type in the striatum are spiny projection neurons (SPNs), which are classically classified into two subtypes: The 'Direct-pathway' neurons, which are supposed to drive action forward ("Go") and the 'Indirect-pathway' neurons that are inhibiting action ("No Go"). However, recent anatomical and functional evidence suggest that this model, while heuristically useful, may need to be modified by incorporating the true phenotypic diversity of striatal SPNs.

For this goal, we have analyzed striatal cells via single cell RNA-seq and observed that up to 30% of SPNs are expressing overlapping D1R and D2R markers. Yet, this population of neurons remain largely ignored, largely due to the lack of a clear genetic definition. Our analyses of SPNs using single cell RNA-seq revealed six discrete subtypes with specific marker genes, which can provide genetic access to these neurons. Moreover, our computational analyses also describe continuous cellular identities within discrete cell subtypes. One of the continua vector within all SPN subtypes shared several genes (particularly Cnr1, Crym, and Wfs1), suggesting a common origin for these vectors. The in situ RNA detection of these vector markers revealed that the expression pattern increases and decreases through dorsal/ventral striatum axis. As a next step, we are addressing how continuous cellular identities are altered through inputs and disorders, in order to identify vectors that relate to the function of a complex tissue.

Functional connectivity in frontal cortex revealed by *in vivo* large-scale network imaging

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Synaptic inhibition orchestrates both spontaneous and sensory-driven activity in the cerebral cortex, and it's generated by interneurons reciprocally connected to other cortical neurons. Interneurons expressing parvalbumin (PV), somatostatin (SOM) and vasoactive intestinal peptide (VIP) are the three largest and non-overlapping classes of interneurons in the mouse cortex. *In vitro* studies have shown cortical interactions between these groups of interneurons and their excitatory partners, but the functional meaning of the connections are poorly understood. To gain further insights into the impact of behavioral state on local cortical circuitry, we employ a novel approach based on resonant scanning 2-photon imaging of large populations of identified cortical neurons in frontal cortex of behaving mice. GCAMP6 calcium sensors were used to image activity of excitatory and inhibitory neurons, using cell type specific CRE-driver lines that also expressed a red fluorescent protein. Neural responses are modulated by brain state, which changes with arousal (excitation), attention, and behavior. To correlate activity pattern of local circuits with brain state, we monitor at the same time ball motion and pupil dilation. It has been shown that pupil fluctuations can track changes in cortical state. Moreover, rapid dilations of the pupil are tightly associated with phasic activity in noradrenergic axons, whereas longer-lasting dilations of the pupil, such as during locomotion, are accompanied by sustained activity in cholinergic axons. Thus, the pupil can be used to sensitively track the activity in multiple neuromodulatory transmitter systems as they control the state of the waking brain.

Our data identify a novel dual role of VIP interneurons to modulate the gain of excitatory neurons. During arousal, pyramidal neurons receive both indirect VIP→SOM cell-mediated disinhibition and direct VIP cell-mediated inhibition. An expected outcome from this circuitry is that variability in the net balance of inhibition and disinhibition generates a heterogeneous response of excitatory neurons, some of which are enhanced during arousal as others are suppressed. The net effect on individual cells is expected to shift their operating point, modulating the gain of pyramidal neurons during arousal. The impact of the PV cells in the local circuitry is still under study, but our findings show that PV cells are functionally heterogeneous and they comprise at least two stable and distinct subnetworks in layer 2/3.

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The ultrastructural sequence of events during developmental remodelling of the *C. elegans* motor circuit

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Animals are born with a nervous system in place to integrate sensory input and effect appropriate behavioural or homeostatic responses. After birth, and throughout the lifetime of the animal, the nervous system undergoes extensive remodelling supporting both developmental maturation and experience-dependent plasticity. This includes synapse formation, elimination and refinement, integration of new neurons into circuits in the form of postnatal and adult neurogenesis, and learning and memory.

Despite the importance of neural circuit remodelling to fundamental biology and human health, the rules and mechanisms controlling synapse specification, formation and elimination in the context of intact and identified neural circuitry are not well understood. This is in part because of the difficulty of visualizing connectivity of a circuit across sequential developmental time points. For this we need serial section electron microscopy (ssEM) of entire neural circuits, which is labour-intensive and low throughput. Although such a dataset is not yet feasible in mammalian animals, recent technical advances have made it possible to perform these experiments in smaller model organisms.

The small invertebrate, *C. elegans*, hatches with a compact nervous system including 22 motor neurons. Within the next 16 hours, post-embryonic neurogenesis gives rise to an additional 55 motor neurons that integrate into the motor circuit. In addition to the synaptogenesis associated with the integration of post-embryonic neurons into existing circuitry, embryonic motor neurons undergo profound synapse formation and elimination events. GABAergic DD-type motor neurons reverse polarity, converting their dendrites into axons and vice-versa. The embryonic role of DD is taken over by post-embryonic VD-type GABAergic motor neurons. Our understanding of this process has been advanced by partial EM reconstruction and imaging of fluorescently tagged synaptic components in conjunction with genetic perturbation of molecular pathways involved in synapse assembly and elimination. However, a full reconstruction of entire neurons across development is required to provide an ultrastructural base for the rich and expanding molecular information.

Here, I will present our work using ssEM across sequential developmental time points to document the cellular events occurring during the remodelling of the *C. elegans* motor circuit. We observe sequential stages of synaptogenesis, which differ in order between embryonic post-embryonic motor neurons. Newly born VD neurons have physical interactions with embryonic neurons that may help to guide neurite extension and synaptogenesis. Both embryonic and post-embryonic neurons form ultrastructurally mature presynapses before extending spine-like extensions from their dendrites into mature postsynaptic fields. These events implicate an elegant mechanism that allows a gradual and seamless transition of the motor circuit to take place without disrupting its functional output.

Tight temporal coupling between synaptic rewiring of olfactory glomeruli and the emergence of odour-guided behaviour in Xenopus tadpoles

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Olfactory sensory neurons (OSNs) are chemoreceptors that establish excitatory synapses within glomeruli of the olfactory bulb. OSNs undergo continuous turnover throughout life, which causes the constant replacement of their synaptic contacts. Using Xenopus tadpoles as an experimental system to investigate synaptic rewiring, we show that immediately after being inserted novel synapses can efficiently transfer information, generating an olfactory-guided behaviour. Upon exposure to waterborne odorants presynaptic terminals of incipient contacts are capable of generating calcium transients that support long lasting depolarizations of olfactory glomeruli. The functionality of rewired terminals relies on well defined readily releasable and a cytoplasmic vesicle pools. Continuous growth of non-compartmentalized axonal processes provides a vesicle reservoir to nascent release sites, which contrasts to the gradual development of cytoplasmic vesicle pools in conventional excitatory synapses. The immediate availability of fully functional synapses upon formation supports an age-independent contribution of OSNs to the generation of odour maps.

A CASPR1/contactin1-containing cellular adhesion complex at retinal ribbon synapses is an early target in experimental autoimmune encephalitis (EAE), a mouse model of multiple sclerosis

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Ribbon synapses in the retina are continuously active glutamatergic synapses with unique presynaptic elements, synaptic ribbons, associated with the active zone. RIBEYE is an essential main protein component of synaptic ribbons. We immuno-isolated synaptic ribbon complexes from the retina with a monoclonal antibody against RIBEYE and found the adhesion proteins CASPR1/contactin1 (CNTN1) as co-immunoprecipitating with RIBEYE. Previously, CASPR1 and CNTN1 have been primarily considered as components of the septate-like junctions in the paranodal region at the nodes of Ranvier and characterized as targets of various autoimmune diseases including multiple sclerosis (MS). In the present study, we demonstrate that the CASPR1/CNTN1-containing adhesion complex is also highly enriched at retinal ribbon synapses close to the synaptic ribbon at a presynaptic site. Interestingly, at that synaptic location the CASPR1/CNTN1 complex is highly sensitive to inflammatory changes in the early, pre-clinical course of experimental autoimmune encephalitis (EAE), a frequently used and well validated mouse model of multiple sclerosis in humans. CASPR1/CNTN1 show strong alterations in synaptic expression at retinal ribbon synapses in EAE and are early targets of an auto-reactive immune system in EAE. We provide evidence that components of the innate immune system rapidly target CASPR1/CNTN1-containing retinal ribbon synapses and trigger structural and functional changes at these synapses.

Dissecting Mouse Retina Development with RNA-seq and CRISPR

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Establishing proper cell circuitry is an important process during neurodevelopment. Few candidates have been implicated in this process as current methods for assessing novel molecules rely on generating conventional germ-line mutants. To circumvent this, we developed a tool that allows us to study neural circuit assembly in vivo through the use of somatic CRISPR mutagenesis. We use this approach to study circuit formation in the mouse retina. In the outer plexiform layer (OPL), axons from rod and cone photoreceptors synapse with dendrites of rod bipolars and cone bipolars, respectively. We used transcriptomic profiling to identify cell surface and secreted proteins expressed in these synaptic partners, and then used CRISPR/Cas9 electroporation to disrupt them in particular cell-types. From these experiments, we found that key players of the Wnt pathway mediate synaptic layer patterning. We found Wnt5a/5b is selectively expressed in rod bipolars and is required for normal OPL. In the absence of Wnt5a/5b an ectopic synaptic layer forms within the Outer Nuclear Layer (ONL), an area normally devoid of neuronal processes. Disruption of the non-canonical Wnt pathway replicates this ectopic OPL phenotype. We demonstrated that Ryk, Fzd4 and Fzd5 are required in rods to suppress the formation of an ectopic neuropil. This suggests Wnt5a/5b signals through Ryk in rods to specify the location of the OPL. In these studies, we demonstrate that this method can be used to characterize a molecular pathway regulating a developmental process such as synaptic layer formation. We envision that this approach can be used to study other developmental questions in the nervous system.

Synaptic Capture: Synapse formation stabilizes filopodial dynamics in *Drosophila* brain development

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Filopodial dynamics are thought to underlie the exploration for molecular cues that direct growth direction or synapse formation. However, the types and roles of filopodial dynamics throughout the development of a living brain are largely unknown. In this study, we are probing the relationship of axonal filopodial dynamics and synapse formation: What are the roles of filopodia in the formation and stability of synapses and how does synapse formation or stability affect filopodial dynamics?

We have recently developed long-term, fast and high-resolution imaging of growth cone dynamics in intact, developing *Drosophila* brains and identified a hitherto undescribed type of filopodia that coincide with synapse formation and characterized by bulbous tips of unknown function[1]. Early factors in synapse formation include Syd-1 and Liprin-α, while Brp (Bruchpilot) is recruited late. Here we show that these markers are differentially recruited to filopodia: Brp^{short}-GFP puncta are never localized to filopodia and form by gradual accumulation in the axon trunk; while Liprin-α can be transiently localized to filopodia. Both Syd-1 and Liprin-α were independently linked to growth cone morphology and layer specificity of R7 axons in the fly visual system[2], raising questions about how synapse formation, filopodial dynamics and axonal targeting are related.

Our live imaging data reveal a multi-step synaptic capture process: First, we hypothesize that filopodial exploration leads to partner identification and bulbous tip formation. Second, Syd-1 functions early in synapse formation; in the absence of *syd-1* almost no active zones mature and the growth cone initiates a dramatically increased filopodial exploration program. Third, Liprin-α is required for synapse maturation; in the absence of *liprin-α*, bulbous filopodia fail to stabilize and growth cones progressively retract from their target layer. Both *syd-1* and *liprin-α* mutant R7 axons have primary defects in active zone maturation that are close to 100% penetrant independent of the growth cone dynamics; in addition, both exhibit completely normal axon targeting and growth cone dynamics until the time point when synapse formation commences. Finally, the 'synaptic' filopodia shorten and mature, Brp-positive active zones are only present on the axon trunk where they remain stable throughout the remainder of development. We conclude that synaptic capture is a multi-step process that stabilizes filopodia and growth cones early during synapse maturation.

(1) Ozel, M.N. et al. (2015). Elife e10721 (2) Holbrook et al. (2012) J. Neurosci 32:18101

Role of SMN in Synapse Maturation

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Spinal Muscular Atrophy (SMA) is a neurodegenerative disease characterized by the loss of spinal cord α-motoneurons, muscle weakness and progressive paralysis. SMN, the defective protein in the disease, participates in mRNA metabolism through snRNPs and mRNPs assembly. In addition, SMN regulates mRNP axonal localization in motoneuron cultures, and fast axonal transport of SMN particles has been observed.

To get a deeper insight into the axonal and presynaptic role of SMN we investigated the distribution and properties of axonal and presynaptic SMN granules and their association with the cytoskeleton in these compartments in control and SMA mouse models. Specifically, we are exploring the association of SMN granules with NFs and MAP1B, a microtubule associated protein involved in neural development.

The study was performed in wild-type and transgenic SMA mouse models, which express full-length and truncated SMN proteins in different amounts. SMN expression was studied by quantitative confocal microscopy at different stages of synaptic maturation.

We identified SMN granules in both motor axons and nerve terminals colocalizing with cytoskeletal elements, such as NF and MAP1B. In addition, we observed that SMN granules disappear in an age-dependent manner in these two compartments, supporting a role for SMN granules in the NMJ maturation.

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Calcium channel auxiliary subunits team up with α-neurexins to regulate Ca^{2+} influx and release

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Synapses mediate the flow of information between neurons via specialized pre- and postsynaptic membrane domains. Voltage-gated calcium channels (VGCCs) are essential for this process because they trigger Ca^{2+}-dependent release of neurotransmitter from vesicles at the presynaptic membrane. We reported earlier that Ca^{2+}-dependent release is impaired when the synaptic cell adhesion molecules α-neurexins (αNrxns) are deleted, pointing to an intricate link between cell-cell recognition/adhesion and neurotransmission. However, regulation of the abundance and activity of VGCCs at synapses, and the possible involvement of Nrxns, are poorly understood. Here, we demonstrate that αNrxn act together with α2δ, auxiliary subunits with roles in trafficking and kinetics of VGCCs, in order to determine the amount of Ca^{2+} influx and vesicle release. Using mostly live imaging with genetically encoded indicators in primary hippocampal neurons from wild-type and mutant mice, we show that deletion of αNrxn reduces synaptic Ca^{2+} transients and vesicle release in knockouts neurons. Furthermore, experiments with inhibitors reveal different contributions from P/Q- and N-type VGCCs to Ca^{2+} influx in WT and KO, or under rescue conditions. These results suggest that synapses depend on αNrxn to reach normal Ca^{2+} influx upon stimulation. Conversely, combined overexpression of αNrxn and α2δ-1 has a strongly facilitating effect, whereas αNrxn alone elevates Ca^{2+} transients only moderately and α2δ-1 alone even reduces Ca^{2+} transients. Interestingly, combined expression of αNrxn with another α2δ subunit, α2δ-3, does not enhance total Ca^{2+} influx, indicating that α2δ subunit-dependent differences may exist in the effect of αNrxn on VGCCs. Finally, data from patch-

clamp recordings of VGCCs expressed in heterologous cells indicate that Nrxn1α mediates a preferential association of α2δ-1 or α2δ-2 with $Ca_V2.1$ and of α2δ-3 with $Ca_V2.2$ pore-forming subunits. Thus, we propose that αNrxn are able to control a specific α2δ subunit-dependent regulation of Ca^{2+} influx through VGCCs, a powerful and novel mechanism to modulate synaptic efficiency.

An essential role for the tetraspanin LHFPL4 in the cell type-specific targeting and clustering of synaptic $GABA_A$ receptors

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Inhibitory synaptic transmission requires targeting and stabilization of $GABA_A$ receptors ($GABA_A$Rs) at synapses. The mechanisms responsible remain poorly understood, and roles for transmembrane accessory proteins, although well described at the excitatory synapse, have not been established at inhibitory synapses. Using molecular, imaging, and electrophysiological approaches, we identify the tetraspanin LHFPL4 as a critical regulator of postsynaptic $GABA_A$R clustering in hippocampal pyramidal neurons. LHFPL4 interacts tightly with $GABA_A$R subunits and is selectively enriched at inhibitory synapses. In LHFPL4 knockout mice there is a dramatic cell type-specific reduction in $GABA_A$R and gephyrin clusters, and the accumulation of large intracellular gephyrin aggregates *in vivo*. While $GABA_A$Rs are still trafficked to the neuronal surface in pyramidal neurons, they are no longer localized at synapses, resulting in a profound loss of fast inhibitory postsynaptic currents. Hippocampal interneuron currents remain unaffected. Our results establish LHFPL4 as a synapse-specific tetraspanin essential for inhibitory synapse function, and provide novel insights into the molecular make-up of the inhibitory PSD.

The identification of Protein tyrosine phosphatase receptor type O (PTPRO) as a synaptic adhesion molecule that promotes synapse formation

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The proper formation of synapses–the specialized unitary structures formed between two neurons–is critical to mediate the information flow in the brain. Synaptic cell adhesion molecules (CAMs) is thought to participate in the initiation of synapse formation process. However, the in-vivo functional analysis demonstrate that most known synaptic CAMs regulate synaptic maturation and plasticity rather than synapse formation, suggesting that either CAMs work synergistically in the process of synapse formation or more CAMs remain to be found. By screening for unknown synaptic cell adhesion molecules (CAMs) using a co-culture system, we revealed PTPRO as a potent CAM that induces the formation of artificial synapse clusters in co-cultures of HEK293T cells and mice hippocampal neurons. PTPRO was enriched in the mouse brain and localized to postsynaptic sites at excitatory synapses. The overexpression of PTPRO in cultured hippocampal neurons increased the number of synapses and the frequency of miniature excitatory postsynaptic currents (mEPSCs). The knockdown of PTPRO expression in cultured neurons by short hairpin RNA (shRNA) reduced the number of synapses and the frequencies of mEPSCs. The effects of shRNA knockdown were rescued by expressing either full-length PTPRO or a truncated PTPRO lacking the cytoplasmic domain. Consistent with these results, the N-terminal extracellular domain of PTPRO was required for its synaptogenic activity in the co-culture assay. Taken together, our data show that the PTPRO is a synaptic CAM serving as a potent initiator of excitatory synapse formation.

Neuronal LRP4 Regulates Synapse Formation in the Developing CNS

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Previous studies have shown that LRP4 expressed in skeletal muscle cells is a key regulator during formation, maintenance and regeneration of the neuromuscular junction (NMJ). Moreover, adult LRP4-deficient brains have cognitive deficits, including hippocampal plasticity, fear-conditioning, associative and spatial learning suggesting important functions in the CNS as well. It has been also described that astrocyte-expressed LRP4 modulates glutamatergic synaptic transmission between neurons indirectly by influencing the release of ATP from astrocytes. However, whether neuronal LRP4 plays a role in the establishment of neuronal circuits by regulating synapse formation or neurite arborization, as observed in the NMJ, was still an open question. Therefore, we investigated in this study the function of LRP4 in embryonic cortical and hippocampal neurons in culture as well as in vivo. We show that knockdown (using synthetic miRNA under the control of the neuron-specific synapsin promoter) of LRP4 in these neurons resulted in fewer synaptic specializations with longer primary dendrites. Viral transsynaptic tracing, a technique that relies on the presence of functional synaptic connections, confirmed a reduced number of presynaptic partner cells in cortical cultures after knockdown of LRP4. This indicates that the reduced number of synaptic specializations after LRP4 knockdown reflected the loss of functional synapses. Moreover, LRP4 overexpression in neurons (using again the neuron-specific synapsin promoter) resulted in the formation of more and shorter primary dendrites with an increased number of spines. Finally we show that knockdown of LRP4 in developing hippocampal and cortical neurons by in utero electroporation of mouse embryos also reduced the number of spines in vivo. Our study represents the first analysis of the function of LRP4 in CNS neurons and our results unequivocally demonstrate, for first time, that neuronal-derived LRP4 is a key regulator of synapse formation in the developing CNS.

Signaling via the adhesion GPCR Latrophilins regulates excitatory synapse formation and specificity in the hippocampus

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Neural circuit assembly requires the orchestration of a precise sequence of molecular events, including axonal pathfinding, target recognition, synapse specification, and molecular organization of the pre- and postsynaptic compartment. Synaptic adhesion molecules are emerging as essential regulators of synapse formation, organization, and specificity. The adhesion GPCR Latrophilins (Lphn) are candidate postsynaptic adhesion molecules with putative signaling capabilities. We found that conditional KO (cKO) of Lphn3 in hippocampal neurons diminished excitatory synaptic strength in a manner that required GPCR signaling. Moreover, the excitatory function of Lphn3 required simultaneous binding to two extracellular adhesion partners, the FLRTs and Teneurins. Expression analysis in vivo revealed that Lphn2 and Lphn3 exhibit distinct patterns along the CA1 pyramidal cell dendritic arbor, with Lphn2 enriched in the stratum lacunosum moleculare, and Lphn3 enriched in the stratum oriens and stratum radiatum. Cell autonomous cKO of either Lphn2 or Lphn3 in CA1 pyramidal cells resulted in deficits in excitatory synapses, suggesting that Lphn2 and Lphn3 may function as postsynaptic recognition molecules that regulate synapse specification from perforant path and Schaffer collateral inputs, respectively. Finally, we observed that postsynaptic sequestration and overexpression of a high affinity, cAMP-specific phosphodiesterase profoundly impaired excitatory synaptic transmission, implicating a general role of compartmentalized GPCR signaling in synapse formation and function. Our results define a novel cell adhesion and signaling pathway mediated by Lphns to regulate synapse formation and specificity.

Post-translational Regulation of Neurexins by Carbonic Anhydrase Related Protein CA10

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Pre- and post-synaptic cell adhesion molecules are believed to play a role in the assembly, validation and plasticity of synaptic connections; a well-known example are presynaptic neurexins and their interactions with diverse post-synaptic ligands. Consistent with a critical role of neurexin-1 in the human brain, exonic deletions affecting the *NRXN1* gene are associated with risk for developing autism spectrum disorder or schizophrenia. The heterozygous patients, as well as experimental data, suggest that *NRXN1* haploinsufficiency is the underlying genetic mechanism. We recently found that the carbonic anhydrase-related protein CA10, an evolutionarily conserved, secreted glycoprotein with unknown function, can bind neurexins in a cis configuration. This interaction can result in the formation of an intermolecular disulfide bond between conserved cysteine residues in neurexins and CA10 to form a stable and stoichiometric complex. Upon forced expression of CA10 in neurons, surface-levels of various neurexin isoforms are specifically enhanced. We are now studying the in vivo function of CA10 as well as the mechanism whereby CA10 can promote neurexin surface-levels. An increased understanding of the posttranslational regulation of neurexins may reveal strategies to counteract molecular imbalances caused by *NRXN1* haploinsufficiency.

POSTSYNAPTIC PROTEOME OF THE HIPPOCAMPAL TRISYNAPITC CIRCUIT

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Advancement of mass spectrometry-based proteomics during the last decade has allowed characterizing the proteome of the nervous system in depth. The proteome of certain sub-cellular structures has received particular attention, especially that of excitatory synapses and its supra-molecular complexes. This effort has resulted in a rather complete atlas of all synaptic components, particularly in mammalian species. Today we can confidently assign a synaptic location to over 4000 different proteins, of which around 2000 localize to the postsynaptic density (PSD). Nevertheless, the spatial resolution of this atlas does not cope with the large cellular and functional heterogeneity of the mammalian brain. To keep advancing in our understanding of the molecular organization of the brain it is indispensible that we can study the synaptic proteome at a microscopic resolution. To achieve this goal we have developed a new method that combines the use of laser-capture microdissection and mass spectrometry-based proteomics to characterize the PSD proteome of microscopic areas of the mammalian brain. We have used this method to establish the postsynaptic proteome of each of the three main hippocampal areas (CA1, CA3 and dentate gyrus) individually, for the first time. We have identified around 2000 proteins in the PSD of each region finding a very large overlap (over 80%) between them. This indicates that the postsynaptic machinery of these hippocampal regions is highly similar despite their different electrophysiological properties. The CA3 region presented the most different PSD proteome with 10% of its proteins being exclusive to this region or highly enriched in it. The CA1 PSD is enriched in well-known signal transmission pathways crucial for glutamatergic synapses, the CA3 PSD is enriched in the protein synthesis and vesicle transport machineries, while the DG PSD is enriched in proteins associated with important neurodegenerative diseases.

Insights into the striatal parvalbumin neurons: towards a specific stimulation of GDNF to protect the nigrostriatal dopaminergic neurons

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The most disabling motor symptoms in Parkinson's disease (PD) stem from the progressive death of the nigrostriatal dopaminergic (DA) neurons. Since there are limited treatment options for PD, neuroprotective agents are currently being tested as a mean to slow disease progression. The use of glial cell line-derived neurotrophic factor (GDNF) held high expectation from preclinical studies, but its exogenous administration in clinical trials proved more problematic. Previous work from our laboratory suggests that stimulating endogenous GDNF may be a valuable strategy to protect the DA neurons. In mice, striatal GDNF is expressed in a restricted population of parvalbumin (PV)-positive interneurons, which form an ensemble of synchronized cells through multiple dendrodendritic electrical synapses. We are seeking a method to specifically increase striatal GDNF and assess its potential as a possible therapy for PD. Our approach is based on the molecular mechanisms that make the striatal (ST) PV interneurons the main GDNF providers, which differ from other PV neuronal population that does not produce GDNF, such as those located in the cortex (CTX). To this end, we used PV reporter mice to capture the tdTomato fluorescent ST or CTX PV interneurons by FACS and run transcriptome analysis. Gene ontology analysis revealed remarkable differences in the intracellular pathways in ST and CTX PV interneurons. Moreover, the comparison of gene expression showed unique receptors and transcription factors that are selectively expressed in PV interneurons of the striatum. These may possibly modulate the striatal GDNF production. The relevance of these specific target genes and pathways is currently under investigation, but these data bring us closer to unravel the cellular/molecular pathways that drive Gdnf expression, and to identify potential pharmacological targets to specifically stimulate striatal GDNF synthesis. The findings of this study could enable us, in the future, to tryout a neuroprotective approach in clinical trials to reduce the degeneration of dopaminergic neurons in PD.

Second genetic hits in synaptic adhesion proteins in autism

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Autism spectrum disorders (ASD) comprise a spectrum of neurodevelopmental disorders characterized by qualitative impairment in communication and social interaction, and restricted and stereotyped patterns of behavior, interests, and activities. ASD show a multifactorial genetic basis, with high genetic heterogeneity. In previous studies, we had identified independent mutations in beta-neurexin-1 (*NRXN1β*) in patients with autism. The ASD-associated mutations led to a decrease in NRXN1β levels and showed partial cosegregation with ASD and other psychiatric traits within the families. Given the polygenic nature of ASD, we aimed at identifying second genetic hits in the families carrying damaging NRXN1β mutations. We performed a genome-wide CNV analysis using the Affymetrix CytoScan HD array. The results were filtered using unbiased criteria based on CNV size (>10Kb), marker density (>1SNP/Kb), and population frequency (MAF>1%) and the selected CNVs were validated by TaqMan analysis. A total of six rare CNVs were identified in four families carrying *NRXN1β* mutations: *IGSF11*, *LRRTM4*, *PTPRD*, *PARK2*, *KATNAL2* and *ATP2C2*. Considering that neither gene function nor expression was taken into account for the CNV selection process, it is remarkable that the genes affected by CNVs or point mutations could be grouped within ASD relevant categories: IGSF11, LRRTM4, PTPRD and NRXN1 proteins participate in synaptic adhesion, while PARK2 is involved in protein ubiquitination. Both pathways have ben previously associated with ASD in candidate-gene or large-scale genome-wide studies. Additionally, NRXN1, LRRTM4 and PTPRD interact at glutamatergic synapses. These results suggest the presence of multiple hits in functionally related genes in individual ASD patients.

Down-regulation of Calcium/calmodulin-dependent serine protein kinase (CASK) disrupts excitatory-inhibitory balance of synapses by down-regulation of GluN2B

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Calcium/calmodulin dependent serine protein kinase (CASK) is a membrane associated guanylate kinase protein (MAGUK), which is associated with nerodevelopmental disorders. CASK is considered to participate in both pre- and postsynaptic functions, but the mechanism and consequences for the brain function has yet to be elucidated, because homozygous CASK-KO mice die before brain maturation. Here, we examined synaptic functions in CASK-KO neurons in acute brain slices of heterozygous CASK-KO female mice. We also analyzed the CASK knock-down (KD) neurons in acute brain slices generated by in utero electroporation. Both CASK-KO and CASK-KD neurons showed a disruption of E/I balance phenotype. We found the expression level of GluN2B was decreased in CASK-KD neurons and the overexpression of GluN2B rescued the disrupted E/I balance in CASK-KD neurons, suggesting that the disruption of synaptic E/I balance was caused by the down-regulation of GluN2B in the CASK deficient neurons.

A unique tyrosine residue in the intracellular domain of neuroligin-1 regulates excitatory versus inhibitory synapse differentiation

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The differentiation of neuronal connections into excitatory or inhibitory synapses is a key question in neurobiology, but the mechanisms which govern the apposition of post-synaptic glutamate and GABA receptors in front of their respective pre-synaptic terminals are unclear. Neuroligins have been shown to participate to synapse development and function by recruiting specific scaffolding molecules such as PSD-95 or gephyrin and receptors such as GABARs, AMPARs and NMDARs. Here, we examined the impact of neuroligin-1 (Nlg1) tyrosine phosphorylation on synapse specification in hippocampal neurons, focusing on a unique intracellular residue (Y782) located in the gephyrin-binding motif. Expression of two Nlg1 point mutants (Y782A versus Y782F) promoted the assembly of functional excitatory and inhibitory synapses, respectively. The Nlg1Y782F mutant blocked excitatory synapse assembly, as did a Nlg1 mutant lacking the PDZ domain binding motif, suggesting that gephyrin and PSD-95 compete for Nlg1 interaction. These effects are specific of Nlg1, since the same mutations in Nlg3 did not alter excitatory synapse formation. Strikingly, optogenetic phosphorylation of Nlg1 at residue Y782 using a photoactivatable tyrosine kinase, induced the formation of new dendritic spines, providing an efficient way to control spine densithy with light. Thus, Nlg1 tyrosine phosphorylation is a crucial switch mechanism that may explain the selective role of the Nlg1 isoform in excitatory synapse development.

ABETA FROM APP/PS1 ALZHEIMER MICE HIPPOCAMPUS INDUCED SYNAPTIC DAMAGE IN VIVO AND IN VITRO

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We aim to investigate the effects of Abeta from young APP/PS1 mouse model of Alzheimer`s disease (AD) on the synaptic integrity, as the loss of synapses strongly correlates with cognitive deficits in patients. Plaque-associated abnormal swellings of neuronal processes represent the first indicator of disease development and might compromise neuronal integrity and synaptic function. Here, we examined the synaptic nature of dystrophic neurites, and the reduction of both synapses and vesicles density in presynaptic terminals along with the progressive accumulation of autophagic structures and Abeta within hippocampal synaptosomes during the aging. We analysed both the direct synaptotoxic effect of plaques in the hippocampus of this model and also the repercussion of the soluble (S1) fraction in neuronal cultures.

Hippocampal synapses were observed under both optic and electron microscopy. Synapses and vesicle density were quantified in periplaque and control (plaque-free) areas by electron microscopy. Primary neuronal cultures were incubated for 48 hours with 6-month-old APP/PS1 and wild-type S1 fractions. In addition, Abeta immunodepletion was carried out with different anti-Abeta antibodies and the levels of synaptic proteins were measured by Western-blot (WB).

Both synapse number and synaptic-vesicles density were significantly decreased in young APP/PS1 mice, close to the Abeta deposits, in several hippocampal layers. Importantly, there was a correlation between the synaptic deficiencies and the distance to plaques, which presented oligomeric forms in their periphery. Some presynaptic elements were abnormally swollen, containing autophagic vesicles. In addition, we found by WB a decrease in several hippocampal synaptic markers as early as 4 months of age in this model, and also in neuronal cultures incubated with S1 fractions. Significantly, the neuronal reduction in VGLUT was reversed after Abeta immunodepletion.

Plaque-associated oligomeric Abeta induced an early deleterious effect on synapses that correlates with memory deficits in young APP/PS1 mice. Moreover, soluble Abeta derived from these transgenic mice reduced synaptic protein content *in vitro*, which was restored after immunodepletion of Abeta species. Therefore, this model produced synaptotoxic Abeta and may represent a valuable tool to test novel treatments to protect synapses as an early therapeutic approach for AD.

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POSTERS

PLD3 role in Alzheimer's Disease and Lysosomes

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Mutations in phospholipase D3 (PLD3) gene increase the risk for Alzheimer's disease (AD). The function of this novel risk gene for AD is poorly understood, nonetheless it was suggested that PLD3 may regulate APP expression. At odd with this mechanistic model, we demonstrate here that PLD3 is not involved in APP processing using a combination of gain- and loss-of-function experiments both in vitro and in vivo. Conversely, we show that PLD3 is localized in late endosomes and lysosomes. Next, to test the relevance of PLD3 in neuronal physiology we generated PLD3 deficient mice. Notably, we showed that deletion of PLD3 impaired lysosomal morphology and caused the accumulation of electron-dense protein waste and lipid droplets. These alterations in lysosomal structure where remarkably similar to the changes observed in tissue from AD patients.

In conclusion, our study convincingly shows that PLD3 mutations do not affect amyloid accumulation and indicates a novel etiological role of PLD3 in the pathology of AD. This work reveals a direct link between PLD3 and lysosomal impairments, highlighting the importance of protein recycling and homeostasis in AD.

Reference:
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Fazzari P, Horre K, Arranz AM, Frigerio CS, Saito T, Saido TC, De Strooper B.
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NEURONAL PENTRAXIN 1 IS ESSENTIAL FOR MITOCHONDRIAL FRAGMENTATION EVOKED BY NEURONAL DEPOLARIZATION AND AMYLOID BETA OLIGOMERS

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Neurons regulate the length of their mitochondria in an activity dependent manner by altering the equilibrium between mitochondrial fission and fusion. However the mechanisms that link neuronal activity with mitochondrial dynamics are not well known. We have investigated the role of Neuronal Pentraxin 1 (NP1), a neuronal activity dependent pro-apoptotic protein, in mitochondrial fragmentation evoked by potassium depolarization or by Amyloid beta (Ab) oligomers in cultured cortical neurons. Both treatments reduce mitochondrial length through different pathways; fragmentation caused by Ab, but not that evoked by potassium depolarization, depends on NMDA receptor activation. In contrast, fragmentation evoked by both treatments is mediated by DRP1. Likewise, NP1 overexpression causes DRP1 dependent mitochondrial fragmentation. Deletion of NP1 inhibits mitochondrial fragmentation evoked either by potassium depolarization or oligomeric Ab and prevents the translocation of DRP1 from cytosol to mitochondria. Moreover, NP1 overexpression does not alter mitochondrial fusion. These results suggest that mitochondrial fragmentation evoked by potassium depolarization or by Amyloid beta (Ab) oligomers is through facilitation of Drp1 dependent mitochondrial fission evoked by NP1.

Developmental changes in synaptic and dendritic structure and function in the mouse barrel cortex

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Synaptic transmission is crucial for neuronal maturation and synaptic function and it relies on the correct apposition of the pre- and post-synaptic density zones. This structural organization is orchestrated by a family of scaffolding proteins (the MAGUKs) that cluster and hold in place the receptors and adhesion proteins required, as well as providing a link to the actin cytoskeleton. PSD95 is highly expressed postsynaptic scaffolding protein in mature glutamatergic synapses. Focusing on key stages around synaptogenesis in the mouse barrel cortex (P5-19), we investigated the changes in PSD95 distribution in a PSD95-eGFP knock-in mouse line. As expected, PSD95-eGFP puncta in the barrel cortex increased over time. The 2-photon imaging of layer-specific distribution of PSD95 revealed an age-specific expression pattern. PSD95 clusters were first strongly detected within the barrels in layer 4, in layer 1 and layer 5A; this was followed by a later increase in layer 2/3. These changes in relative levels of fluorescence in the different layers mirror the spatiotemporal sequence of the formation of synapses during development in this area, as thalamocortical inputs reach the cortex. We also investigated the emergence of dendritic spikes during the same key stages. Dendritic spikes are branch-specific ion channel-mediated events that have been shown to contribute to neuronal computation by boosting synaptic input and provide the neuron with spatial and temporal information of incoming inputs. Most studies have focused on their role in the adult mouse brain and their effects on neuronal and animal behaviour, but not much is known about dendritic spikes during development. We have examined dendritic spikes by inducing them in layer 4 spiny stellate neurons before, during and after synaptogenesis to investigate a possible instructive role during this delicate process.

Study of pathogenic mutations from human NBS (Nicolaides Baraitser Syndrome) patients

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Neurological diseases represent a large group of disorders. For many of them, the causal factors, either genetic or environmental, are poorly understood. Recent advances in large-scale DNA sequencing and genome wide association study (GWAS) help to narrow down the candidate pathogenic genes.

We are interested in the neurological disease named Nicolaides Baraitser Syndrome (NBS) with causal *SMARCA2* mutations recently identified in human patients. The tight genetic linkage made this disease a good system to study the molecular mechanism of the pathological processes, which may also be applied to other mental disorders sharing common clinical features with NBS.

We are generating the *SMARCA2* mutant KI lines and Dox-inducible lines in human H1 ES cells. We want to recapitulate the causal effects and study the molecular mechanism of the mutations in ES and neural progenitors, as well as in neurons differentiated from these KI and inducible lines. Both morphological and functional assays will be performed to dissect the defects from these mutant neurons. We will further investigate at molecular level how the *SMARCA2* mutations can affect their protein functions within BAF complexes, including chromatin structure remodelling and genome wide gene expression regulation. With confirmed result from culture system, we plan to generate KI mice to perform the behavioural studies for *in vivo* functions of these mutants.

Ketamine differentially affects anhedonic, resilient and control mice in chronic unpredictable stress paradigm

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Depression is one of the most common diseases in the world and it is characterized by different symptoms such as anhedonia, depressed mood, and increased anxiety.

Ketamine - a NMDA receptors antagonist - has gained wide attention as a potential fast acting antidepressant. Remarkable efficacy of ketamine in alleviating depression symptoms has been documented by several animal studies. Ketamine half-life is about 2,5 h whereas its effects are shown to be detectable hours later (up to 7 days) (Ma et al., 2013). This suggests that ketamine may only trigger pathways governing antidepressive behaviors. In humans and in animal models chronic as well as acute severe stress may precipitate or exacerbate depression in majority (50-70%) of individuals but not all, the remaining individuals are called resilient. The aim of our study was to evaluate the antidepressive effect of ketamine on the behavior of resilient and susceptible to depression mice in chronic unpredictable stress paradigm and correlate this effect with changes in structural plasticity of dendritic spines.

We show that ketamine injection causes different behavioral effects in anhedonic compared to resilient mice. We demonstrate that ketamine increases anhedonia in resilient animals and significantly decrease immobility in FST only in anhedonic mice what correlates with significant increase in serum corticosterone levels 36h post injection. Finally, we discuss morphological changes in dendritic spines associated with ketamine effect and state of depression in animals.

Analysis of behavioral, molecular and morphological drug-induced changes both in anhedonic and resilient mice may be particularly informative for distinguishing drug- induced therapeutic symptoms.

Flow cytometric analysis of mouse brain synaptosomal fraction reveals loclisation of β-dystroglycan on double synapses

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One of the basic functionalities of a neuronal network is the synaptic plasticity. Synaptic plasticity is regulated not only through neurotransmitter release and membrane ion channel gating but also through ECM modifications. Among proteases secreted in the vicinity of the synapses, that can trigger proteolytic ECM modification is MMP-9. This enzyme has multiple functions in the brain being critical for learning and memory formation. The current model of synaptic plasticity postulates that MMP-9 is being released in response to neuronal stimulation and specifically cut transsynaptic proteins such as β–dystroglycan.

Among unclear aspects of the MMP-9 mode of action is the type of synapse that this protein is secreted on. It has been well established that MMP-9 activity is associated with excitatory synapses, however one of its most specific substrates namely β–dystroglycan usually co-localize with inhibitory (GABAergic) synapse markers. Here we show results that may shed light on this discrepancy. We analyzed isolated mouse synaptosomes from P2 fraction and analysed them using flow cytometry after staining them with antibodies against several synaptosomal markers including gephyrin, psd-95 and SNAP-25. We found out that β-DG is present on small subset of synaptosomes (5-10%) that exhibit expression of both post-synaptic markers (psd-95 and gephyrin). These results indicate that β-DG is associated with specific population of spines, i.e. double synapse spines, which have two different inputs – one excitatory and the other inhibitory.

Proteolytic cleavage of ECM elements cause release of biologically active factors and take active part in learning and long-term memory creation processes. Thus understanding the role of ECM elements in synaptic plasticity is one of the top priories of modern neuroscience.

The autism mutation R704C in neuroligin-3 affects social recognition and social memory in mice, and fluoxetine reverses the social deficits

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Neuroligins are a family of postsynaptic cell-adhesion molecules encoded by four genes (NL1–NL4), all of which have been implicated in autism. The R704C mutation, identified in the NL4 gene of a patient with autism, is the only single-point autism-related mutation located in the NL protein's cytoplasmic region. We have studied the molecular effect of this mutation by introducing it into NL3, and have generated NL3 R704C knock-in (KI) mice. Although the synaptic effect of the R704C mutation has been studied in multiple systems, the behavior of NL3 R704C KI mice has not been analyzed. Here, we examined behaviors of NL3 R704C KI mice that are relevant to the symptoms of autism. Compared to wild-type mice, NL3 R704C KI mice spent more time in self-grooming and showed impairments in motor learning, reciprocal social interaction, and social novelty preference; these characteristics are comparable to autistic behavioral traits. We also found that fluoxetine but not MPEP or oxytocin recovered the impairment in social novelty preference in the NL3 R704C KI mice. Likewise, fluoxetine but not MPEP or oxytocin restored the reduced AMPAR–mediated synaptic transmission observed in the hippocampal CA1 region of these mice. These results suggest that reduced AMPAR function is linked to the pathophysiology of social deficits in NL3 R704C KI mice.

SYNAPTIC CIRCUITS ASSOCIATED WITH SOCIAL INTERACTION ARE AFFECTED IN AN AUTISM MOUSE MODEL EXPRESSING A NEUREXIN-1 BETA MUTANT

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Social cognition is an important human skill frequently impaired in several neurodevelopmental disorders, including autism. Human genetic approaches have led to the identification of a number of genes associated with autism. However, how specific brain areas relevant for social interaction are affected by mutations in autism-associated genes is not known.

To identify synaptic circuits relevant to social interaction, we have used the three-chamber social test. In the three-chamber social arena the tested mouse performs social and non-social interactions with animated and unanimated caged-stimuli, respectively. We have modified the three-chamber test to record local field potentials in selected brain areas of behaving mice. The modifications did not affect the behavior of the tested mice, as shown by increased interaction of control mice with the social stimulus. Interestingly, electrophysiological recordings in control mice showed a differential pattern of activity during interaction with social versus non-social stimulus in selected brain areas of the cortex and striatum. Selectively, social interaction increased delta oscillations in prefrontal cortex and striatum. To validate these findings, we performed similar recordings in behaving βNrx1ΔC mice, a validated mouse model of autism that expresses a dominant negative beta-neurexin mutant in principal neurons of the cortex and striatum (1). In contrast to control mice, βNrx1ΔC mice do not show preferential interaction with the social stimulus in the three-chamber test. Notably, the electrophysiological signature induced by the interaction with the social stimulus in control mice was impaired in βNrx1ΔC mice. Furthermore, using co-immunoprecipitation experiments we found that synaptic defects of βNrx1ΔC mice affect glutamatergic synapses mediated by neuroligin-1, a beta-neurexin binding partner.

Our data suggest that characterization of synaptic circuits and electrophysiological activity during social interaction in mice may help to understand the mechanisms responsible for autistic-like behavior. This knowledge may contribute to identify molecular mechanisms for future interventions in autism.

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HERC1 ubiquitin ligase is essential for evoked neurotransmitter release and optimal propagation of electrical signals at the mouse neuromuscular junction

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HERC1 E3 is an ubiquitin ligase involved in membrane traffic and widely expressed in mammalian tissues. It belongs to the ubiquitin-proteasome system (UPS), which play and important role in protein degradation and cell homeostasis. A missense mutation in HERC1 in the *tambaleante* (*tbl*) mice provokes loss of Purkinje cells in the cerebellum, tremor, and unstable gait. Recently, we discovered that, before the cerebellar degeneration takes place, the *tbl* mice suffer from a reduction in the number of vesicles available for release at the neuromuscular junction (NMJ) (Bachiller et al., 2015). By using a multidisciplinary approach, the aim of the present work was to study to which extent the alteration in HERC1 E3 affect other cells in the nervous system and how this may influence the motor dysfunction observed in these mice. Morphological analysis of glial cells in motor axons revealed an impairment in axonal wrapping and myelin sheath thickness, together with alteration in non-myelinated Schwann cells at the level of the NMJ in *tbl* mutant mice. The functional analysis show a consistent delay in the propagation of the action potential in mutant mice in comparison with control littermates. At high stimulation frequency, the propagation velocity became progressively further reduced along the train. Altogether, the present data show that HERC1 is important for the normal maintenance of neuronal and glial cells in the nervous system.

The HIF1α-PHD3 pathway mediates microglial dysfunction in Alzheimer's disease

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Microglia, the brain innate immune cells, react against amyloid-ß peptide (Aß) plaques in Alzheimer's disease (AD). Microglial dysfunction is a key process in the pathophysiology of AD but the molecular pathways involved remain unknown. We describe the hypoxia-inducible factor (HIF) as a main driver of microglial dysfunction. HIF1α accumulates in the hippocampus of AD patients and we show that the microenvironment around Aß plaques is hypoxic in AD mice. HIF1α-regulated transcripts are the most enriched gene set in microglia in two different AD models. Prolyl hydroxylase 3 (PHD3) is highly up-regulated through HIF1α and its absence prevents a microglial antiviral response also observed in AD models. Moreover, PHD3 modulates the expression of *Trem2* and *Cd33*, two risk genes for AD. Functionally, PHD3 deficiency modifies the activation of microglia, increases the microglia mediated shielding from Aß plaques and restores cognitive deficits in an AD model. This novel role of the HIF1α/PHD3 pathway in microglial dysfunction provides new insights into the mechanisms of neurodegeneration.

BEHAVIORAL DEFICTS, HIPPOCAMPAL NEURONAL LOSS AND EARLY MORTALITY IN TRANSGENIC MICE EXPRESSING THE PRESENILIN SUBSTRATE NEUREXIN-CTF

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Presenilins (PS1, 2) are the active subunit of the gamma-secretase complex that cleaves a growing number of plasma membrane proteins. PS function is required to maintain neurotransmitter release at glutamatergic terminals and inhibition of PS genes in forebrain glutamatergic neurons of PS cDKO mice produces memory deficits and age-dependent neurodegeneration (1). Because inactivating mutations in *PS* genes cause early-onset familial Alzheimer's disease (FAD), the identification of proteins responsible for the PS loss-of-function effects has clinical relevance. Neurexins (Nrxns) are presynaptic plasma membrane proteins involved in formation and function of synaptic contacts. We showed that Nrxns are synaptic substrates for PS (2). Following ectodomain shedding, membrane-bound Nrxn C-terminal fragment (Nrxn-CTF) is processed by PS. Nrxn-CTF accumulates in cells expressing FAD-linked *PS1* mutations and in presynaptic terminals of PS cDKO mice at an early developmental time coinciding with synaptic deficits (2). These data suggest that inhibition of the proteolytic processing of Nrxn can mediate synaptic and behavioral deficits associated to loss of PS function.

Using deletion mutants, we identified the cleavage site that generates Nrxn-CTF. To analyze the role of the proteolytic processing of Nrxns *in vivo* without broadly affecting PS activity toward other substrates, we generated transgenic mice that express a HA-tagged Nrxn-CTF protein in forebrain glutamatergic neurons in an inducible-manner. Biochemical experiments showed that HA-Nrxn-CTF accumulates at presynaptic terminals, mimicking the distribution of endogenous Nrxn-CTF in PS cDKO mice. Interestingly, Nrxn-CTF mice showed decreased body weight and increased early mortality. Morphological analysis showed decreased brain volume and neuronal loss in the hippocampal region. Behavioral analysis showed increased locomotor activity in Nrxn-CTF mice.

Together, our data indicate that inhibition of Nrxn processing by PS is deleterious to glutamatergic function, resulting in behavioral deficits, and suggest that accumulation of Nrxn-CTF by FAD-linked PS mutations may contribute to the physiopathology of Alzheimer's disease.

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Morphology of functional spines formed in the absence of presynaptic glutamate release

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Dendritic spines are the major transmitter reception compartments of glutamatergic synapses in most principal neurons of the mammalian brain, and play a key role in the function of nerve cell circuits. The formation of functional spine synapses is thought to be critically dependent on presynaptic glutamatergic signaling. We analyzed the spatial frequency and size distribution of dendritic spines that have been formed in absence of presynaptic glutamatergic signaling in CA1 pyramidal neurons in mutant hippocampal slice cultures. The findings demonstrate that formation and maintenance of dendrites and functional spines are independent of synaptic glutamate release.

Synapse heterogeneity and its implication in Spinal Muscular Atrophy

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During development, synapses undergo structural and functional changes. Physiologically, the expression of synaptic proteins follows a spatial-temporal pattern, which leads to a great molecular synaptic diversity. This could determine the different functional properties of each synapse (for example, the differences between fast and slow firing muscles). Besides that, synaptic heterogeneity may contribute to the selective muscular vulnerability found in certain neuromuscular disorders, like Spinal Muscular Atrophy (SMA).

The aim of our work is to investigate possible molecular players that determine the functional characteristics of several muscles during development and to examine whether the molecular synaptic signature is related to the differential synaptic vulnerability in a mouse model of SMA. We investigate the origin and the potential mechanisms responsible for the molecular changes in different synapses in both physiological and pathological conditions by combining quantitative confocal microscopy, protein quantification by immunoblotting and electrophysiology.

Our results demonstrate that synaptotagmin (Syt) experiences a switch between its isoforms 1 and 2 at the NMJ. At this synapse, Syt1 is expressed during the first stages of development and is replaced by Syt2, the main isoform in mature synapses. However, the speed of this process varies among different muscles. In contrast, synaptic vesicle protein 2B (SV2B) doesn't change during development, although SV2A isoform disappears in fast muscles.

In motor nerve terminals of a mouse model of Spinal Muscular Atrophy (SMA), the most frequent genetic cause of infant mortality, we found a large and specific reduction in the expression of Syt2. Likewise, we found a direct correlation between the motoneuron pool vulnerability and the time course of the Syt1 downregulation. We are now studying if calmoduline (CaM), a calcium binding protein implicated in the expression of distinct synaptic proteins, could be altered in SMA.

These results indicate that the NMJ synapses are molecularly heterogeneous and that this might determine the selective vulnerability in different pathological processes.

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CONDITIONAL ELIMINATION OF CYSTEINE STRING PROTEIN-α IN PARVALBUMIN-POSITIVE INTERNEURONS IN VIVO

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Synapses are plastic structures that properly support the operation of neural circuits from birth to senility. Our laboratory investigates the molecular mechanisms underlying the maintenance of synapses along time in the mammalian brain. Cysteine String Protein-alpha (CSP-alpha) is a DNAJ-containing synaptic vesicle protein that, as a co-chaperone, prevents presynaptic degeneration. In humans, mutations in the DNAJC5 gene, that codes CSP-alpha, cause autosomal-dominant adult onset neuronal ceroid lipofuscinosis that leads to seizures and early death in young adults. CSP-alpha KO mice undergo early lethality within 1-2 months postnatally. Interestingly, in the absence of CSP-alpha, neurons operating at high firing rates, such as parvalbumin-positive (PV+) interneurons suffer from activity-dependent presynaptic degeneration. The early lethality of CSP-alpha KO mice has, so far, impaired investigations to understand molecular mechanisms and secondary circuit dysfunctions. Now, we have used genetically modified mouse stem cells bearing a DNAJC5 floxed allele (European Conditional Mouse Mutagenesis Program, Skarnes et al. Nature. 474:337-42 (2011)) to generate CSP-alpha conditional knock-out mice. We have observed that, in CSP-alpha$^{fl/fl}$ mice, CSP-alpha expression is specifically abolished by Cre-recombinase activity. Importantly, those results validate the genomic manipulation at the DNAJC5 locus to generate conditional knock-out mice. Next, we have bred CSP-alpha$^{fl/fl}$ mice against PV^{Cre} knockin mice (129P2-Pvalb$^{tm1(cre)Arbr}$ /J) that express Cre recombinase in PV+ neurons and with mice that express fluorescent reporters and optogenetic actuators following exposure to Cre recombinase (YFP and channelrhodopsin-2/tdTomato fusion protein). Interestingly, these novel mouse lines, develop a significant neurological phenotype that, however, does not lead to the early lethality observed in conventional CSP-alpha KO mice. Such a phenotype opens novel perspectives to investigate the molecular mechanisms connecting synaptic degeneration with long term neuronal dysfunctions. We expect our approach will help to understand the functional response of neural circuits to synaptic deficits in PV+ positive interneurons.

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