UKE
HAMBURG

41st

Blankenese Conferences

June 22-25
2024
Hamburg

SYNAPTOPATHIES

Molecular Mechanisms of Brain Disease

Abstract Book

JUNG-Stiftung
FÜR WISSENSCHAFT & FORSCHUNG
AUSGEZEICHNETE HUMANMEDIZIN

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University Hospital Hamburg-Eppendorf (UKE)

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Organizing Institutions / Patronage
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JUNG-Stiftung für Wissenschaft & Forschung - Ausgezeichnete Humanmedizin, Hamburg

Contents

Blankenese Conferences since 1979

The Blankenese Conference series was initiated in 1979 by the late Gebhard Koch and Dietmar Richter, eminent biochemists/molecular biologists at the University Hospital Hamburg-Eppendorf (UKE). Since then, the meetings have been organized whenever possible on a yearly basis. In 2004 Gebhard Koch retired as a chair and until 2023 Wolfgang Meyerhof from the German Institute of Human Nutrition in Potsdam replaced him as co-chair of the Blankenese Conferences. In 2019 Eckart Gundelfinger from the Leibniz Institute for Neurobiology in Magdeburg accepted the invitation to serve as additional chairperson helping to organize the Conferences in the years to come.

The founders of the Blankenese Conferences in 1979

The Conferences are held at the Elsa-Brändström-Haus, a venue beautifully situated above the river Elbe in the Blankenese area of the Hanseatic City of Hamburg. Previous conferences covered topics in biochemistry, molecular and cellular biology, genetics, neurobiology, virology, and molecular medicine. At these meetings, topics of great current interest are discussed bringing together key investigators from different areas, hence promoting the exchange of ideas by leading scientists who may rarely meet at other symposia. The size of the conferences is limited to around one hundred participants, which creates a stimulating atmosphere for scientific exchange. Leading scientists from all over the word are invited to lecture on their fields of interest and scientists early in their career are particularly encouraged to attend and to contribute with their latest results. Posters and brief oral presentations from selected applications complement the lectures by the invited speakers.

At a special conference “Reflection on Forty Years of Blankenese Conferences: Signaling in Health and Disease” in 2019, speakers from almost all previous meetings were brought together to recapitulate 40 years of scientific development in the fields covered by the conference series.

Many internationally recognized scientists from the UKE, the Center for Molecular Neurobiology of Hamburg University (ZMNH) as well as from neighboring and collaborating Universities and Research Institutes have contributed as co-organizers to the thematic diversity and the success of the Blankenese Conferences. Major financial support for the Conferences over the years came from different resources such as the City State of Hamburg, the Deutsche Forschungsgemeinschaft (DFG), the Federal Ministery of Education and Research (BMBF), the European Commission, Human Frontier Science Program Organization as well as various Foundations including Volkswagen Foundation, Thyssen Foundation, Hertie Foundation or Ritz Foundation in addition to numerous other sponsors.

Starting from 2024 the UKE and the Jung-Stiftung für Wissenschaft und Forschung have assumed patronage of the Blankenese Conference series and cover significant parts of the budget. A steering committee associated with the Dean’s Office of the Medical Faculty at the UKE was established to ensure a successful continuation of the Blankenese Conferences.

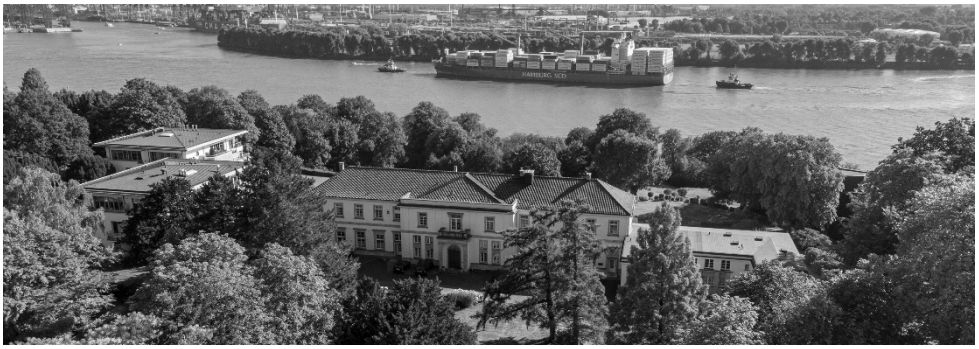
The Jung Foundation

The Jung Foundation for Science and Research, based in Hamburg, Germany, annually provides up to three awards in recognition of fundamental and advanced research projects of significant clinical relevance. To date, the foundation has invested more than 15 million euros in supporting researchers whose projects build a bridge between research and the bedside. Under the motto of 'Excellence in human medicine', the foundation makes a significant contribution to the development of new treatment methods. The Jung Prize for Medicine, the Jung Gold Medal for Medicine and the Jung Career Advancement Award for Medical Research are among the most highly endowed medical prizes in Europe. With the additional awarding of fellowships and German scholarships, the foundation provides a total funding of up to 650,000 euros annually.

Throughout their careers, the winners of the Jung Prize for Medicine have received other important awards as well. Three of them have even been awarded a Nobel Prize: Prof. Dr Rolf Martin Zinkernagel in 1996, Prof. Dr Harald zur Hausen in 2008 and Prof. Emmanuelle Charpentier in 2020. There are also numerous laureates of the Jung Foundation among the important ERC Advanced Grants: Prof. Peter Carmeliet, Prof. Ulrich Hartl, Prof. Ivan Dikic, Prof. Stefanie Dimmeler, Prof. Jens Brüning, Prof. Gary Lewin and Prof. Ruth Ley as well as Prof. Christian Büchel in 2011 and in 2020 and Prof. Tobias Moser in 2015 and in 2022.

Once a year, the Jung Foundation honors its award winners in an award ceremony. In 2024, this festive ceremony took place on a small scale for the invited Jung family at the beginning of May. In addition to the award ceremony, the Jung Symposium has been held since 2021, at which the award winners present their excellent research in interesting lectures. Since 2023, the symposium has been held as hybrid event. Participants are able to follow the lectures worldwide via livestream and also be present in the Ian K. Karan lecture hall at the University Medical Center Hamburg-Eppendorf.

For the first time, in 2024, the Jung Foundation is sponsoring the Blankenese Conferences. In this year represented by esteemed past Jung Prize recipients Professor Dr Thomas Jentsch (Jung Prize for Medicine 2001) and Professor Dr Reinhard Jahn (Jung Prize for Medicine 2006 and member of our Scientific Advisory Board 2011-2019), the Jung Foundation is excited to contribute to the exchange of knowledge among medical professionals.



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SCIENTIFIC PROGRAM

Saturday, June 22, 2024

12:30	Registration and check-in at the Elsa-Brändström Conference Center
Opening Session #1	
15:00-15:15	Opening remarks **Eckart D. Gundelfinger** / **Dietmar Richter** (Chair persons of the Blankenese Conferences)
15:15-15:30	Welcome address **Blanche Schwappach-Pignataro** (Dean of the University Medical School Hamburg and Member of the Board of Trustees, JUNG-Stiftung)
JUNG-lecture (1) 15:30-16:10	**Reinhard Jahn** *Exocytosis of synaptic vesicles - from molecules to function*
Session #2	(co-sponsored by the **GBM Study Group Molecular Neurobiology**) Chairs: Jörg Bartsch / Stefan Kins
16:15-16:45	**Tobias M. Böckers** *Excitatory Synapses and Neuropsychiatric Diseases*
16:50-17:20	**Torben Hausrat** *Tubulin isotypes: specific functions in the brain?*
17:25-17:55	Coffee break
17:55-18:25	**Nils Brose** *Dynamic regulation of presynaptic function and plasticity in health and disease*
18:30-19:00	**Gerhard Schratt** *Non-coding RNA function in neural circuit development and behaviour*
19:05-19:20	**Maaike A. van Boven** (*Short Communication*) *Timing is of the essence: desynchronized synaptic transmission as novel disease mechanism in SYT1-associated disorder*
19:30	Welcome Dinner

Sunday, June 23, 2024

Session #3	(co-sponsored by the DFG-Research Unit **Syntophagy**) Chairs: Michael R. Kreutz / Anna Karpova
9:00-9:30	**Natalia Kononenko** *Nurturing brain health: the role of autophagy in neuronal metabolism*
9:35-10:05	**Sandra-Fausia Soukup** *How is autophagy regulated at the synapse? Consequences in brain health and disease*
10:10-10:40	**Michael A. Cousin** *Synaptic vesicle endocytosis dysfunction and neurodevelopmental disorders*
10:45-11:00	**Sebastian Samer** (*Short Communication*) *Protein transport from synapse to nucleus is essential for the maintenance of CREB activity after induction of synaptic plasticity*
11:05-13:00	**Poster Session (Posters #1 - #18)** with Coffee served at posters
13:00-14:00	Lunch Break
Session #4	"A Neurochemist's View on Functions and Dysfunctions of Brain Synapses" (sponsored by the **International Society for Neurochemistry (ISN)**) Chairs: Michael Cousin / Eckart Gundelfinger
14:05-14:35	**Anne-Sophie Hafner** *Excitatory presynapse dysfunction in Alzheimer's disease*
14:40-15:10	**Isabel Pérez Otaño** *Synaptic machinery for lasting synaptic changes – GIT1/mTOR complexes control synapse-specific translation and memory*
15:15-15:45	**Eunjoon Kim** *NMDA receptor dysfunction in mouse models of autism spectrum disorders*
15:50-16:15	Coffee Break
16:15-16:45	**Etienne Herzog** *A synaptomic analysis reveals dopamine hub synapses in the mouse striatum*
16:50-17:20	**Peter Penzes** *Synaptic ectodomain signaling and neurodevelopmental disorders*
17:25-17:40	**Hans-Jürgen Kreienkamp** (*Short Communication*) *Variants in LRRC7, encoding Densin-180, lead to intellectual disability, autism, aggression and abnormal eating behaviors*
JUNG-lecture (2) 17:45-18:25	**Thomas Jentsch** *Control of vesicle size – an unsuspected role of the 'novel' acid-activate Cl channel ASOR*
18:30-19:50	Dinner
Session #5	"Immune system-related Synaptopathies" Chair: Manuel Friese
19:50-20:20	**Michela Matteoli** *Immune-synaptopathies: how the immune system affects synapse function*
20:25-20:55	**Harald Prüss** *Autoimmunity and neurodegeneration -- new disease concepts*

Monday, June 24, 2024

Session #6	(including **EMBO Young investigator Lecture**) Chairs: Matthias Kneussel / Dietmar Kuhl
9:00-9:30	**Thomas Bourgeron** *The genetic architecture of autism: from medicine to neurodiversity*
9:35-10:05	**Marina Mikhaylova** (GBM-supported talk) *SHANK3 in parvalbumin neurons*
10:10-10.40	**EMBO YIP Lecture** **Cecile Charrier** *Molecular mechanisms of synaptic development: insights from human-specific genes*
10:45-11:00	**Alberto Catanese** (*Short Communication*) *Multiomics analysis of hiPSC-derived motoneurons identify pathological synaptic signatures commonly shared across the heterogenous spectrum of Amyotrophic Lateral Sclerosis*
11:05-13:00	**Poster Session (Posters #19 - #36)** with Coffee served at posters
13:00-14:00	Lunch Break
Session #7	"Young Women in Science" Chairs: Cecile Charrier / Ora Ohana
14:00-14:30	**Katarzyna M. Grochowska** *Lysosomal control of the metastable proteome regulates structural spine plasticity*
14:35-15:05	**Cordelia Imig** *Dissecting molecular mechanisms underlying serotonin release from sensory cells in the gut epithelium in health and disease*
15:10-15:40	**Noa Lipstein** *Presynaptic mechanisms in neurodevelopmental and neurodegenerative brain disorders*
15:45-16:00	**Marilyn Tirard** (*Short Communication*) *Effects of Perturbed Sumoylation, Neddylation, and Ufmylation on Synaptic Transmission*
16:05 -16:30	Coffee Break
Session #8	Chairs: Reinhard Jahn / Jacob B. Sørensen
16:30-17:00	**Josef T. Kittler** *Molecular mechanisms of inhibitory synapse formation and plasticity*
17:05-17-45	**Matthijs Verhage** including an introduction to **SynGO** *Disease mechanisms and intervention strategies for SNAREopathies*
17:50 -18:30	**Morgan Sheng** *Synaptic and molecular mechanisms underlying schizophrenia and bipolar disorder*
18:45	Dinner (Awarding of **Poster Prizes**)
20:00	Evening event (Round table)

TALKS

(Sat. 15:30)
Jung Lecture (1)

Reinhard Jahn
Max Planck Institute for Multidisciplinary Sciences Göttingen, Germany

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Exocytosis of synaptic vesicles - from molecules to function

(Sat. 16:15)

Tobias M. Böckers
Ulm University, Germany

Excitatory Synapses and Neuropsychiatric Diseases

(Sat. 16:50)

Torben Hausrat

UKE Hamburg, Germany

Tubulin isotypes: specific functions in the brain?

(Sat. 17:55)

Nils Brose
Max Planck Institute for Multidisciplinary Sciences Göttingen, Germany

Dynamic regulation of presynaptic function and plasticity in health and disease

(Sat. 18:30)

Gerhard Schratt
ETH Zürich, Switzerland

Non-coding RNA function in neural circuit development and behaviour

(Sat. 19:05)
Short Communication

Maaike A. van Boven
VU Amsterdam, Netherlands

Timing is of the essence: desynchronized synaptic transmission as novel disease mechanism in SYT1-associated disorder

(Sun. 09:00)

Natalia Kononenko
University of Cologne, Germany

Nurturing brain health: the role of autophagy in neuronal metabolism

(Sun. 09:35)

Sandra-Fausia Soukup

Bordeaux Neurocampus, France

How is autophagy regulated at the synapse? Consequences in brain health and disease

(Sun. 10:10)

Michael A. Cousin
University of Edinburgh, United Kingdom

Synaptic vesicle endocytosis dysfunction and neurodevelopmental disorders

(Sun. 10:45)
Short Communication

Sebastian Samer
LIN Magdeburg, Germany

Protein transport from synapse to nucleus is essential for the maintenance of CREB activity after induction of synaptic plasticity

(Sun. 14:05)

Anne-Sophie Hafner
Radboud University Nijmegen, Netherlands

ISN International Society for Neurochemistry

Excitatory presynapse dysfunction in Alzheimer's disease

(Sun. 14:40)

Isabel Pérez Otaño
University Miguel Hernández Alicante, Spain

ISN International Society for Neurochemistry

Synaptic machinery for lasting synaptic changes – GIT1/mTOR complexes control synapse-specific translation and memory

(Sun. 15:15)

Eunjoon Kim
KAIST Daejeon, South Korea

ISN International Society for Neurochemistry

NMDA receptor dysfunction in mouse models of autism spectrum disorders

(Sun. 16:15)

Etienne Herzog
Bordeaux Neurocampus, France

ISN International Society for Neurochemistry

A synaptomic analysis reveals dopamine hub synapses in the mouse striatum

(Sun. 16:50)

Peter Penzes

Northwestern University Chicago, United States of America

ISN International Society for Neurochemistry

Synaptic ectodomain signaling and neurodevelopmental disorders

(Sun. 17:25)

Hans-Jürgen Kreienkamp
UKE Hamburg, Germany

Variants in LRRC7, encoding Densin-180, lead to intellectual disability, autism, aggression and abnormal eating behaviors

(Sun. 17:45)
JUNG-lecture (2)

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Thomas Jentsch
FMP Berlin, Germany

Control of vesicle size – an unsuspected role of the 'novel' acid-activate Cl channel ASOR

(Sun. 19:50)

Michela Matteoli
Humanities University Milan, Italy

Immune-synaptopathies: how the immune system affects synapse function

(Sun. 20:25)

Harald Prüss
Charité Berlin, Germany

Autoimmunity and neurodegeneration -- new disease concepts

(Mon. 09:00)

Thomas Bourgeron

Institute Pasteur Paris, France

The genetic architecture of autism: from medicine to neurodiversity

(Mon. 09:35)

Marina Mikhaylova
Humboldt University Berlin, Germany

SHANK3 in parvalbumin neurons

(Mon. 10:10)
EMBO YIP Lecture

Cecile Charrier

École Normale Supérieure Paris, France

Molecular mechanisms of synaptic development: insights from human-specific genes

(Mon. 10:45)
Short Communication

Alberto Catanese
UKE Hamburg, Germany

Multiomics analysis of hiPSC-derived motoneurons identify pathological synaptic signatures commonly shared across the heterogenous spectrum of Amyotrophic Lateral Sclerosis

(Mon. 14:00)

Katarzyna M. Grochowska
LIN Magdeburg, Germany

Lysosomal control of the metastable proteome regulates structural spine plasticity

(Mon. 14:35)

Cordelia Imig
University of Copenhagen, Denmark

Dissecting molecular mechanisms underlying serotonin release from sensory cells in the gut epithelium in health and disease

(Mon. 15:10)

Noa Lipstein
FMP Berlin, Germany

Presynaptic mechanisms in neurodevelopmental and neurodegenerative brain disorders

(Mon. 15:45)
Short Communication

Marilyn Tirard
Max Planck Institute for Multidisciplinary Sciences Göttingen, Germany

Effects of Perturbed Sumoylation, Neddylation, and Ufmylation on Synaptic Transmission

(Mon. 16:30)

Josef T. Kittler
University College London, United Kingdom

Molecular mechanisms of inhibitory synapse formation and plasticity

(Mon. 17:05)

Matthijs Verhage
VU Amsterdam, Netherlands

Disease mechanisms and intervention strategies for SNAREopathies

(Mon. 17:50)

Morgan Sheng
Broad Institute Cambridge, United States of America

Synaptic and molecular mechanisms underlying schizophrenia and bipolar disorder

POSTER ABSTRACTS

1) *Functional characterization of the disease-associated scaffold protein CNKSR2*

Poornima Anantha Subramanian[1,2], Hanna Zieger[3], Benno Kuropka[4], Taanisha Gupta[1], Judith von Sivers[1,2], Stella-Amrei Kunde[1], Nils Rademacher[1], Sarah Shoichet[1,2]

[1]Charite Universitätsmedizin, Germany; [2]Einstein Center for Neurosciences, Berlin, Germany; [3]University of Bordeaux, France; [4]Institute of Chemistry and Biochemistry, Freie Universität Berlin, Germany

Connector Enhancer of Kinase Suppressor of Ras-2 (CNKSR2 or CNK2), also known as membrane-associated guanylate kinase-interacting protein-1 (MAGUIN), is a multidomain scaffold protein that is predominantly expressed in the brain, with enriched expression at the postsynaptic density (PSD) of excitatory synapses. Our overarching aim is to understand the functional role of the CNK2 scaffold molecule at these synapses. We have identified novel interaction partners of CNK2 and explored the functional interaction between CNK2 and specific regulatory kinases (see Zieger et al., 2020); in current work, we are also exploring the functional interactions between CNK2 and synaptic scaffold molecules. We are also pursuing studies on several disease-associated CNK2 mutations that result in the truncation of the amino acids towards the C-terminal region Specifically, in preliminary unpublished studies, we observe that the disease-associated truncation mutation resulting in a transcript that codes for 712 of 1034 amino acids is associated with altered spine morphology, suggesting that the missing C-terminal region might be important for proper regulation of the scaffold function of CNK2 in dendritic spines. We have taken advantage of this disease-associated truncation mutant to map regions of CNK2 that are important for specific protein-protein interactions, and specifically address how the C-terminal region of CNK2 might regulate the scaffolding function of the protein through the modulation of interactions with other proteins. In a comparative mass spectrometry (MS)-based approach, we identified proteins from crude synaptosomes that preferentially interact with either the wild-type or the truncated version of CNK2. We have identified several differentially interacting proteins and are now exploring the functional implications of these interactions with a focus on dendritic spine morphology and dynamics.

2) *Disease mutations of SNAP25 Isoleucine192 identify different SNARE complex stoichiometries for spontaneous and evoked release*

Victoria Amstrup Vold[1], Jie Yang[2], Maiken Østergaard[1], Anna Schrøder Lassen[1], Anna Kádková[1], Alexander M. Walter[1], Yongli Zhang[2,3], Jakob B. Sørensen[1]

[1]Department of Neuroscience, University of Copenhagen, Blegdamsvej 3B, 2200 Copenhagen N, Denmark; [2]Department of Cell Biology, Yale School of Medicine, New Haven, CT 06511, USA; [3]Department of Molecular Biophysics and Biochemistry, Yale University, New Haven, CT 06511, USA.

SNAREopathies are complex neurodevelopmental syndromes that arise from disease mutations in the individual protein components of the synaptic transmission machinery. We investigated two such mutations in the SNARE protein SNAP25: I192N and I192T. These two mutations displayed different degrees of severity in the clinic with I192N causing lethality within the first year of life and I192T leading to moderate developmental delay. Single-molecule experiments identified a graded destabilization of the C-terminal end of the SNARE complex and reduced binding to the Munc18:VAMP2:syntaxin acceptor complex, with I192N more impaired than I192T similar to clinical observations. When expressed in SNAP25 knockout neurons, both mutants reduced spontaneous and evoked release as well as vesicular release probability while increasing paired-pulse ratios, again with I192N more severely affected than I192T. Only I192N decreased neuronal survival both with and without co-expression of wild-type SNAP25. In wild-type neurons, I192N was dominant-negative with a stronger effect on spontaneous than evoked release and mildest for sustained release. Using modeling, we show that these findings can be explained by the number of SNARE complexes that must engage in each release mode: the 6 SNARE complexes that partially form during vesicle priming affect spontaneous release whereas full assembly of 3 of the 6 complexes drives evoked release. In a heterozygous patient, the effect of the disease mutant is partly counteracted by increasing the number of complexes required for evoked release (from 3 to 4 or 5), rendering evoked release partly resistant to mutations.

3) *Characterisation of Magi-family synaptic scaffolding proteins in human iPSC derived neurons*

Maximilian Borgmeyer[1], Julia Knocks[2], Doris Lau[1], Lukas Einhäupl[2], Tomas Fanutza[3], Christian Wozny[3], Nina Wittenmayer[1,2]

[1]Institute for Translational Medicine, Medical School Hamburg, Germany; [2]Institute of Anatomy, Brandenburg Medical School, Brandenburg an der Havel, Germany; [3]Institute for Institute for Molecular Medicine, Medical School Hamburg, Germany

Synapse formation is critical for the wiring of neural circuits in the developing brain. The composition and function of synapses has predominantly been studied in rodent cells. Here we show a novel protocol to generate human iPSC-derived forebrain-like neurons that show rapid synaptic development. To achieve this, we use an astrocyte feeder layer attached to a complex mix of ECM-components. Neuronal stem cells (NCS) are plated on this layer for maturation into neurons. We use this protocol to provide an initial characterisation of Magi-family synaptic scaffolding proteins in human neurons. Both Magi1 and Magi2 are expressed in the brain, however expression levels of Magi-2 are much higher in neurons than in any other cell type, while Magi-1 is expressed more evenly throughout cell types and tissues. In rodents the synaptic scaffolding protein S-SCAM/Magi-2 plays a critical role in the assembly and maintenance of synapses and interacts with signalling proteins facilitating synapse to nucleus signalling. Increased expression of S-SCAM/Magi-2 in the human brain is associated with schizophrenia. We show that Magi-1 can be found at both inhibitory and excitatory synapses in human neurons and can substitute for the loss of Magi-2. Lastly, we analyse how S-SCAM/Magi-2 levels at the synapse influences gene expression, via synapse to nucleus signalling.

4) Synaptotagmin-7 splice variants are differently involved in dense-core vesicle secretion in mouse chromaffin cells

Joana Martins[1], Sebastien Houy[1], **Mario Carvalho[2]**, Ralf Mohrmann[3], Jakob Sørensen[4]

[1]Department of Neuroscience, University of Copenhagen, Denmark; Currently: FUJIFILM Nordic Denmark, Copenhagen, Denmark; Department of Neuroscience, University of Copenhagen, Denmark; Currently: FUJIFILM Nordic Denmark, Copenhagen, Denmark; [2]Department of Neuroscience, University of Copenhagen, Denmark; [3]Institute for Physiology, Otto-von-Guericke University, Magdeburg, Germany; [4]Department of Neuroscience, University of Copenhagen, Denmark

Synaptotagmin-7 (Syt-7) is one of the main calcium sensors for large dense-core vesicle fusion in adrenal chromaffin cells, and it is involved in facilitation in central neurons. This protein consists of a single transmembrane domain and two Ca2+ and phospholipid-binding C2 domains, C2A and C2B, separated by a linker region. This linker is alternatively spliced in several isoforms of Syt-7. Two splice variants are expressed in mouse chromaffin cells: synaptotagmin-7 beta (Syt-7 beta) and the more abundant shorter isoform synaptotagmin-7 alpha (Syt-7 alpha). Syt-7 beta differs by having an insertion of 44 amino acids within the linker region. We report that Syt-7 beta supports a larger primed pool and more total secretion than Syt-7α. We verified this by performing electrophysiological measurements on adrenal chromaffin cells from Syt-7 knockout mice virally expressing Syt-7 alpha and Syt-7 beta. These differences cannot be explained by changes in linker size or calcium-dependence of secretion. Nevertheless, we observed that the Syt-7 beta-mediated increase in secretion is sensitive to the inhibition of protein kinases. Indeed, the alternatively spliced linker region contains putative phosphorylation sites. These data indicate that vesicle priming/unpriming may be regulated by the alternative splicing of Syt-7, and that this process may depend on the phosphorylation of Syt-7 linker region.

5) *Multiomics analysis of hiPSC-derived motoneurons identify pathological synaptic signatures commonly shared across the heterogenous spectrum of Amyotrophic Lateral Sclerosis*

PD Dr. Alberto Catanese

Institute of Anatomy and Cell Biology, Ulm University

The recent advances in techniques and mathematical methods increased our understanding of the genetic basis of ALS, as several novel genes have been linked to this disease either causally or as risk factors. This significantly helped in gaining more knowledge into the extremely heterogeneous pathological landscape of this fatal disease, but the exact molecular pathomechanisms characterizing it have been barely clarified. In particular, a fundamental question remains unanswered: what are the crucial and core commonalities characterizing the different ALS cases that, together with the genetic background and pathogenic mutations, contribute to the onset and progression of this pathology?
To address this question, we combined multi-omics approaches with hiPSC-derived models and post mortem samples to clarify the pathological convergencies defining ALS. By using this experimental strategy, we could show a progressive loss of synaptic contacts linked to altered CREB-dependent transcription in hiPSC-derived motor neurons (Catanese et al., 2021). These alterations were matched by a progressive loss of electrophysiological properties, and all these phenotypes could be rescued along with neuronal survival by enhancing synaptic activity using K+ channel blockers (Catanese et al., 2021; Sommer et al., 2022). These results put the synapse under a different perspective: instead of representing a vulnerable cellular structure undergoing alterations during neurodegeneration, it appears as a crucial hub actively contributing to disease progression. Based on these observations, we could show that the loss of synaptic transcripts represents a common signature shared by major ALS genes: C9orf72, SOD1, FUS and TARDBP. Interestingly, this phenotype anticipates neuronal death in vitro, is matched by altered methylation patterns characterizing the promotor regions of synaptic genes in hiPSC-derived motor neurons, and significantly correlates with the transcriptional landscape of post mortem spinal cord samples (Catanese et al., 2023). This evidence supported our vision of the synapse as a crucial, druggable structure involved already at the early phases of neuronal degeneration. In fact, replenishment of the presynaptic composition was sufficient to rescue neuronal survival in all the mutants we considered in our approaches (Aly et al., 2023).

6) *Experience-dependent regulation of neuronal and synaptic development by the human-specific gene FRMPD2B*

Eirini Christopoulou*, Joseph Zamith* and Cécile Charrier
*Contributed equally to this work

Institut de Biologie de l'ENS (IBENS), Ecole Normale Supérieure, CNRS, INSERM, Université PSL, 75005 Paris, France

The advanced cognitive abilities that characterize our species are tightly linked to the staggering complexity of the human neocortex. Human cortical neurons are morphologically more complex than those of other species. Their dendrites are more ramified, they form more synaptic connections and develop over longer time scales. These features reflect human-specific regulations of neuronal morphogenesis and synaptic development. Yet, their underlying molecular mechanisms remain largely unknown. Here, we characterize the role of FRMPD2B (FERM and PDZ Domain containing 2B), a human-specific gene that is the product of a partial gene duplication that occurred during the transition between Australopithecus and Homo. FRMDP2B is almost exclusively expressed in cortico-projecting excitatory neurons of the neocortex during the period of neuronal morphogenesis and synaptogenesis, but its role has never been investigated. Using targeted in utero electroporation, to sparsely express FRMPD2B in mouse L2/3 cortical pyramidal neurons, we demonstrate that it increases dendritic branching and spine density, with corresponding changes in excitatory synaptic transmission. Additionally, FRMPD2B regulates axonal development, by increasing axonal branching and presynaptic terminals in cortical L5, indicating that FRMPD2B-expressing neurons receive and send more information. Furthermore, we show that FRMPD2B is expressed in an experience- and activity-dependent manner. Together, our results reveal novel mechanisms through which a human-specific modifier partakes in experience-dependent expansion of cortico-cortical connectivity, opening new avenues to understand the unique complexity of the human brain.

7) *Disease variants in the C2A and C2B domains of Synaptotagmin-1 produce different synaptic phenotypes in IPSC derived human neurons.*

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Synaptotagmin-1 (Syt1) is the Ca2+ sensor in the synapse essential for synchronization of neurotransmitter release with the action potential (AP). Recently, missense variants in Syt1 have been associated with severe intellectual disability, movement disorders, behavioral disturbances and EEG abnormalities. Patients carrying these mutations are all diagnosed with Syt1-associated neurodevelopmental disorder, although strong heterogeneity exists in severity of symptoms. Overall, patients with mutations in the C2B domain of Syt1 seem to be more severely affected than patients with C2A mutations. To investigate the cellular basis for this domain dependence we created homozygous human IPSC lines for five disease variants at different locations in Syt1: two in the C2A domain at locations outside the Ca binding pocket (E209K, P216R), two in the Ca binding pocket of C2B (I368T, N371K), and one at the site that forms the primary interface of C2B with the SNAREs (P401L). Using autapse electrophysiology we found a stark reduction in AP evoked excitatory postsynaptic currents (EPSCs) in the C2B variants only, while no effect was found for the C2A variants. Interestingly, short-term synaptic plasticity during 40Hz train stimulation was similar for all variants, except for the N371K which showed strong facilitation. Cumulative release at the end of the train was not different between any of the groups, except for the P401L mutation, which showed a reduction. Furthermore, spontaneous release was increased in the E209K variant and showed a trend to higher frequencies in the P401L variant, but not for the other groups. This first description of Syt1 patient variants in human neurons shows that C2B variants have stronger cellular phenotypes which correlates with stronger disease severity for these patients.

8) *Investigating local regulators of presynaptic proteostasis in midbrain dopaminergic boutons*

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Dopamine (DA) is an essential neurotransmitter regulating motor and limbic functions released by midbrain dopaminergic (mDA) neurons. Due to the excessively branched morphology and their high pacemaking activity, mDA neurons are particularly sensitive to oxidative stress impaired degradation of misfolded proteins. DA releasing boutons require tightly regulated and efficient local control of proteostasis. MDA boutons co-release glutamate and, although having different morphologies and slightly different release machineries, share many components of presynaptic vesicle trafficking and degradation systems with glutamatergic boutons. The active zone protein Bassoon and E3 ligases Parkin and Siah1 are essential components controlling proteostasis in glutamatergic presynapes. Since Bassoon and Parkin, among other proteins of the autophagy, endolysosomal and proteasomal pathways, are candidate genes for neurodegenerative syndromes including Parkinson disease (PD) and progressive supranuclear palsy (PSP), we aim to understand their roles in degradation pathways in mDA boutons. Using a conditional knockout system for dopaminergic neurons, we investigate changes in clearance system levels and parkinsonian phenotypes under loss of the local regulator Bassoon (B2D cKO). Our findings show that in mDA neurons, loss of Bassoon leads to enhanced somatic levels of autophagy marker RFP-LC3. Interestingly, we found reduced levels of endogenous tyrosine hydroxylase (TH) in both the soma and boutons of these neurons. Consistent with this, immunoblot data of striatal fractions of B2D cKO showed reduced TH levels. Furthermore, we aim to utilize the induction of presynaptic autophagy by loss of Bassoon to gain insights into the proteins involved in regulation of presynaptic mDA autophagy such as E2 and E3 ligases. Understanding the regulation of presynaptic autophagy is particularly important for developing therapeutic targets for the treatment of PD and other synaptopathies.

9) *The inhibitory function of neuroserpin is not required for correct brain development and synaptic plasticity*

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The serine protease inhibitor neuroserpin (Ns) is critically involved in the pathophysiology of neurological, neuropsychiatric and neurodegenerative disorders. Ns expression is restricted to the nervous system and particularly prominent in all brain regions at late stages of neuronal development. In the adult brain it is restricted to regions related to learning and memory, and is rapidly upregulated after neurological insults, e.g. ischemic stroke. The mechanism of action of Ns is still unclear: Whereas Ns involvement in the inhibition of the serine protease tissue plasminogen activator (tPA) has been demonstrated, Ns function has been shown to be partially independent from its inhibitory role.

Using Ns-deficient mice, we investigated the role of Ns in development and maintenance of the nervous system, and found premature termination of developmental neurogenesis in the hippocampus. In juvenile animals, during synaptic maturation, we observed altered morphology and extracellular matrix composition of hippocampal synapses. Furthermore, in adult animals, absence of Ns led to a decrease in synapse density and synaptic potentiation in the hippocampus, deficits resulting in social and cognitive alterations. To elucidate both inhibitory and non-inhibitory mechanisms, we generated a knock-in mouse model expressing a non-inhibitory variant of Ns unable to bind the target protease. The mouse is viable and fertile. Mutant Ns is synthesized in the brain at levels similar to its wild-type counterpart. Moreover, histological examination of the brain shows no gross alterations in brain structure and normal Ns expression pattern in the hippocampus. Comparing phenotypic alterations in the knock-in mouse with those observed in the absence of Ns revealed that Ns itself, and not its function as a serine protease inhibitor, is required for correct brain development and synaptic plasticity.

10) GIT1/ βPIX complexes target mTORC1 to synaptic sites

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De novo protein synthesis is indispensable for neural circuit rewiring and stable memory encoding through synaptic modifications. However, the regulation of protein synthesis at the synaptic level remains elusive due to the extensive compartmentalization of neurons. Our previous work identified a neuronal signaling complex composed of the postsynaptic scaffold GIT1, the p21-activated protein kinase exchange factor beta (βPIX) and the mechanistic target of rapamycin complex 1 (mTORC1) that couple synaptic stimuli to mTOR-dependent protein synthesis. We further showed that non-conventional NMDA receptors with GluN3A subunits act as critical negative regulators of mTORC1 signaling by competing for GIT1 binding to mTOR (Conde-Dusman et al., eLife, 2021). Here we investigated the molecular determinants of synaptic mTORC1 localization and signaling. We find that GIT1 and βPIX promote the targeting of mTOR to synapses. Conversely, silencing GIT1 or βPIX inhibits synaptic mTOR localization and activation in primary cultured neurons. Disrupting the formation of GIT1:βPIX condensates by expressing mutants unable to bind each other or unable to form macromolecular condensates also reduced mTOR recruitment to synaptic spines. Our findings reveal GIT1:βPIX condensates as essential platforms for targeting mTOR signaling to synapses. More generally, our work supports the concept of membrane-less biomolecular condensates as a mechanism for concentration of synaptic signaling machinery.

11) Investigating the pathophysiological and molecular underpinnings of SynGAP Syndrome.

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SynGAP Syndrome is a severe neurodevelopmental disorder (NDD) caused by mutations in the SYNGAP1 gene. It is typically characterized by moderate to severe intellectual disability, autistic features, and frequent seizures, there is currently no adequate therapy for affected individuals. The SynGAP1 protein is a synaptically enriched GTPase activating protein (GAP). Early studies on the function of SynGAP1 using mouse KO disease models highlighted an upregulation of the Ras-ERK signal cascade and corresponding increase in the phosphorylation and exocytosis of AMPA receptors in the SynGAP1 heterozygotes, providing a putative mechanistic explanation for the observed network hyperexcitability in patients. However, recent studies highlight that Ras-ERK mediated upregulation of AMPAR exocytosis is not the only mechanism underlying the disease. In this project, we aim to contribute to our understanding of the multifaceted role of SynGAP1 in neuropsychiatric disorders. First of all, we are establishing biochemical and cell-based assays that enable us to investigate and compare the function of different disease-associated point mutations by assessing their effects on the Ras signal cascade. Through analysis of human mutation data, we have identified regions of the protein that seem to be especially important for function (disease-associated missense mutations are especially common in the GAP and C2 domains), and we will investigate how defects within these domains influence signalling. We have also taken advantage of a comparative quantitative mass spec approach to identify novel synaptic Ras interactors that may likewise be affected by altered function of SynGAP1. Using fixed and live-cell imaging approaches, we are exploring how these proteins – together with Ras – can influence factors that control neuronal excitability. The ultimate objective is to uncover molecular pathways that are regulated by SynGAP1.

12) *Aberrant neuronal connectivity and network activity in a patient-derived model of the developing cortical microcircuitry in Schizophrenia Spectrum*

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Schizophrenia spectrum (SCZ) is a synaptopathy affecting about 1% of the population with a highly variable representation of symptoms. Here, we present an induced pluripotent stem cell (iPSC)-based model derived from patients with SCZ. iPSCs were differentiated into glutamatergic and GABAergic neurons by overexpression of the transcription factors NGN2 and Ascl1+Dlx2, respectively. Transcriptome analysis revealed pronounced upregulation of synaptic genes in both cell types, suggesting intrinsic alterations of synapse formation as a major disease phenotype.
As an imbalance of excitation and inhibition in the cortical microcircuitry is hypothesized to account for network dysfunction in SCZ, we established a defined co-culture of glutamatergic and GABAergic neurons (E-I co-culture) to study this aspect. The spontaneous calcium activity of glutamatergic and GABAergic neurons was increased in E-I co-cultures. In parallel, a decrease of the length of axon initial segments was observed for both cell types, possibly due to adaptive shortening in response to increased neuronal activity. In addition, we show increased formation of excitatory synapses targeting GABAergic neurons and elevated EPSC frequency in GABAergic neurons, suggesting increased excitatory drive onto inhibitory neurons. In turn, increased activity of GABAergic neurons might result in elevated inhibitory drive onto excitatory neurons. Accordingly, we observed a downregulation of EPSC frequency in glutamatergic neurons. Finally, MEA recordings revealed increased network synchronization in SCZ, possibly related to altered GABAergic drive.
Overall, our results obtained with the co-cultures suggest a shift in E-I balance due to altered synaptic connectivity and neuronal activity, which might contribute to aberrant network activity in SCZ. These findings add to our observation of increased neuroinflammatory phenotypes and dopaminergic dysregulation in iPSC-derived neurons derived from the same SCZ patients.

13) *Myosin VI controls localization of Golgi satellites at active presynaptic boutons*

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Neurons, as long-living non-dividing cells with complex morphology, depend on a highly elaborate secretory trafficking system which ensures the constant delivery, removal and recycling of proteins and membranes. Previously, we have shown that simplified Golgi-related structures called Golgi satellites (GS), distinct from the somatic Golgi complex, are present in dendrites of primary hippocampal neurons and are involved in glycosylation and local forward trafficking of membrane proteins. However, whether GS are also targeted to axons of principal neurons has not been explored. Here, we investigate the subcellular distribution of GS in adult hippocampal neurons and discover that mobile and stationary GS are present along the entire axonal length, extending to the distal tips of the growth cone. Live imaging experiments revealed that neuronal firing modulates the switch between long range transport mediated by kinesin and dynein and stalling. We found that GS frequently pause or stop at pre-synaptic sites in an activity-dependent manner. This behavior depends on the acting cytoskeleton and the actin-based motor protein myosin VI. Our study demonstrates that neuronal activity can dynamically regulate the organelle trafficking in neurons. Furthermore, we hypothesize that similarly to dendrites, axonal GS may be involved in various processes, including protein processing and delivery to lysosomes and autophagosomes.

14) *Molecular mechanisms of spinogenesis in the context of reward-regulated learning*

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Within the reward circuit, the medium spiny neurons (MSN) of the striatum receive both dopaminergic and glutamatergic projections. Dopamine inputs encode for reward and error prediction reward, while glutamatergic afferences give information about the environmental context. MSN are thus involved in reward-regulated learning and drugs of abuse such as cocaine interfere with that physiological learning process.
Our team has shown that upon cocaine administration, dopamine and glutamate receptors D1R and NMDAR act synergistically to activate the ERK pathway in MSN, thereby regulating gene expression, epigenetic modifications and neuronal plasticity. The increase in spine density induced by cocaine is also ERK dependent, furthermore, we have shown that spine growth and maintenance are both controlled by ERK albeit through distinct molecular mechanisms. Whilst spine growth relies on ERK but not translation, spine maintenance is supported by ERK-activated MNK1 and translation. Therefore, our aim is to identify the cytosolic target of ERK involved in cocaine-induced spine growth in MSN, and thus to unravel the molecular mechanisms of spinogenesis. Among the ERK targets, WAVE2 seems to be a potential candidate. WAVE2 is a protein of the WASP family that belongs to the WAVE Regulatory Complex which positively regulates the ARP2/3 complex involved in actin nucleation. WAVE2 is targeted by ERK on 4 phosphorylation sites, and mutation of these sites causes the loss of lamellipods in cell lines.
Here, we confirmed that WAVE2 is phosphorylated by ERK in cultured MSN. Furthermore, WAVE2 was localized in dendrites and spines and the increase in dendritic spine density induced by a dopaminergic agonist in cortico-striatal co-culture was prevented in the presence of an inhibitor of the Arp2/3 complex.

15) *Presynaptic Filopodia: decoding a new frontier of structural plasticity and its impact on epileptogenesis.*

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Our research challenges the widely held belief that synapses, the tiny structures where neurons communicate, remain structurally unchanged over long periods. Contrary to this notion, we've discovered rapid and transient changes in the synaptic structure in response to neuronal activity. This structural plasticity is driven by changes in the organization of the actin cytoskeleton. As a result, small protrusions called filopodia emerge and recede on the presynaptic side, where neurotransmitters are released.

Intriguingly, synaptic vesicle (SV) fusion occurs locally at the tip of the filopodia, which also establish connections with postsynaptic structures and enhances the neuronal wiring plasticity during action potential discharge. These changes in the presynaptic structural dynamics were revealed via the simultaneous monitoring of SV fusion or synaptic calcium spikes, with high-resolution optical imaging in both primary hippocampal cultures and acute brain slices. These changes were confirmed using ‘zap-and-freeze’ electron microscopy, in which the ultrastructure of these synapses was captured with unprecedented detail, milliseconds after stimulating them.

Furthermore, we found that these filopodia affect the mechanics of neurotransmitter release. Specifically, the emergence of filopodia sustains membrane tension at release sites, influencing synchronous release of neurotransmitter. Inhibiting the formation of filopodia reduced neurotransmitter release rates, indicating their importance in synaptic function.

Importantly, our findings extend beyond basic neuroscience. By modulating these structural changes, we were able to reduce the severity of seizures in experimental models. This suggests that targeting these mechanisms could hold promise for treating conditions characterized by excessive neuronal activity, such as epilepsy.

In summary, our study uncovers a novel synaptic mechanism with therapeutic promise for disorders marked by abnormal neuronal activity.

16) *Functional internally tagged Vps10p-Domain receptors: A novel tool to investigate their endosomal itineraries, dimerization and ligand interactions that reveals their potential role in BDNF transport*

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The Vps10p-domain (Vps10p-D) receptor family consists of Sortilin, SorLA, SorCS1, SorCS2, and SorCS3. They mediate internalization and intracellular sorting of specific cargo in various cell types, but underlying molecular determinants are incompletely understood. Deciphering the dynamic intracellular itineraries of Vps10p-D receptors is crucial for understanding their role in physiological and cytopathological processes. However, studying their spatial and temporal dynamics by live imaging has been challenging so far, as terminal tagging with fluorophores presumably impedes several of their protein interactions and thus functions. Here, we addressed the lack of appropriate tools and developed functional versions of all family members internally tagged in their ectodomains. We predict folding of the newly designed receptors by bioinformatics and show their exit from the endoplasmic reticulum. We examined their subcellular localization in immortalized cells and primary cultured neurons by immunocytochemistry and live imaging. This was, as far as known, identical to that of wt counterparts. We observed homodimerization of fluorophore-tagged SorCS2 by coimmunoprecipitation and fluorescence lifetime imaging, suggesting functional leucinerich domains. Through ligand uptake experiments, live imaging and fluorescence lifetime imaging, we show for the first time that all Vps10p-D receptors interact with the neurotrophin brain-derived neurotrophic factor and mediate its uptake, indicating functionality of the Vps10p-Ds. In summary, we developed versions of all Vps10p-D receptors, with internal fluorophore tags that preserve several functions of the cytoplasmic and extracellular domains. These newly developed fluorophore-tagged receptors are likely to serve as powerful functional tools for accurate live studies of the individual cellular functions of Vps10p-D receptors.

17) Variants in LRRC7, encoding Densin-180, lead to intellectual disability, autism, aggression and abnormal eating behaviors

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Members of the leucine rich repeat (LRR) and PDZ domain (LAP) family of proteins are essential for early animal development and histogenesis. Densin-180, encoded by LRRC7, stands out as the only LAP protein selectively expressed in neurons. It acts as a postsynaptic scaffold at glutamatergic synapses, linking cytoskeletal elements with signalling proteins such as the α-subunit of Ca2+/calmodulin-dependent protein kinase II (αCaMKII). We identify 33 individuals with a dominant neurodevelopmental disorder due to heterozygous missense or loss-of-function variants in LRRC7. The clinical spectrum involves intellectual disability, autism, ADHD, aggression and, in several cases, hyperphagia-associated obesity. A variant in the PDZ domain interferes with synaptic targeting of Densin-180 in primary cultured neurons. LRR variants disrupt binding to newly identified interaction partners including protein phosphatase 1 (PP1), thereby interfering with its capacity to act as scaffold for phosphatase/kinase signalling. We conclude that LRRC7 encodes a major determinant of intellectual development and behaviour.

18) *Developing a pipeline for the study of epigenetic autism risk genes in C. elegans*

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University of Southampton, Gerald Kerkut Trust, John W Caddick Scholarship.

Autism Spectrum Disorder has a complex genetic architecture, and the presentation of sensory differences is a diagnostic feature. C. elegans provides a model to investigate genetic determinants of autism, facilitated by sensory assays that underpin complex behaviours. Almost 50% of the high confidence genes documented by The Simons Foundation Autism database (SFARI) are gene expression modifiers. In our approach we have established a bioinformatic pipeline for the selection of gene expression modifiers documented by SFARI for investigation in C. elegans. Genes were selected based on gene function, presence of orthologues in C. elegans and viable putative null strain availability. Supplementary information was gathered on each candidate such as gene expression patterns in humans and C. elegans as well as known protein interactors to inform future experiments. 52 candidate genes were identified for investigation of sensory processing architecture, development, and behavioural changes.
jmjd-3.1, a candidate gene identified by our pipeline is a lysine demethylase and orthologue of the mammalian gene KDM6B. Putative null jmjd-3.1 strains have impaired attractive and aversive chemosensory processing. Mechanosensory responses and gross sensory anatomy does not appear to be disrupted. jmjd-3.1 identifies complex epigenetic modulation of sensory and sensory integrative behaviour. Assays identified here show clear neuronal underpinnings and will be extended to the remaining candidates identified by the bioinformatic pipeline. Comparison of phenotypes that emerge in the different sensory modalities will inform if epigenetic modulators converge on defined determinants of synaptic and neuronal function. This will develop the understanding of how pleiotropic regulation of gene expression underpins the emergence of neuroatypical behaviour that signature autism.

19) Tau Protein: Neuronal Functions and Dysfunctions

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Tau protein has functions in axon growth, synaptic activity, and dysfunctions in Alzheimer disease (AD). "Abnormal" Tau allows monitoring AD by higher levels in brain, "hyperphosphorylated" tau peptides in CSF, or aggregated tau by brain imaging. The appearance of aggregates has been proposed to arise from trans-cellular spreading of tau aggregates. Efforts of AD treatments are mostly aimed at reducing tau protein, phosphorylation, or aggregation. Here we describe our recent studies of tau w.r.t. aggregation, phosphorylation, and drug treatment of mice and discuss some open issues.
(a) Spreading of aggregation is typically tested by transfecting cell models with short XFP labeled tau constructs, exposing them to tau from AD brain, and observing cellular peaks of fluorescence, interpreted as aggregates. By contrast, our experiments showed that XFP reporters inhibit bona fide amyloid interaction, implying that the spots in cells reflect local inclusions but not proper fiber assembly.
(b) Using tg mice expressing pro- or anti-aggregant tau in the transentorhinal region, we found that tau spreading to the hippocampus does not depend on a disease-causing mutation.
(c) Mice with pro-aggregant tau were tested with several drugs for effects on cognitive decline. While known inhibitors of in vitro aggregation did not halt disease progression, blocking adenosine A1 receptors achieved complete reversal of decay, arguing that cognitive decline is not caused by tau aggregation as such.
(d) The view of "hyperphosphorylated AD tau" stems from early studies showing that tau from AD-patients post mortem was highly phosphorylated, contrary to normal controls. However, recent analysis by native mass spectroscopy showed cellular tau to be constitutively hyperphosphorylated similar to AD tau. The discrepancy arises because PPases are active post mortem so that soluble tau is dephosphorylated, while P-sites within tau aggregates are protected.
Support: DZNE, MPG, Cure Alzheimer's Fund

20) *Reduction in synaptic vesicle glycoprotein 2A (SV2A) in Alzheimer's disease (AD) and temporal lobe epilepsy (TLE)*

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Synaptic vesicle glycoprotein 2A (SV2A) is a presynaptic vesicular protein and a target for anti-seizure drugs. The function of SV2A in synapse biology has not been ruled. The concentration of SV2A has been considered a biomarker for synaptic density, and this has attracted clinical interest because SV2A binding sites can be determined in vivo using imaging technology. In this study, autoradiography with the selective SV2A radiotracer [3H]UCB-J has been used to determine the level of SV2A binding in the cerebral cortex in two cohorts of patients with AD and TLE, respectively, compared with two age-matched control groups. According to transcriptome data, SV2A mRNA is expressed in all glutamatergic and GABAergic neuronal subpopulations in the human cerebral cortex. The binding level of SV2A is lower in the cerebral cortex in the TLE patients compared to the control. Further, the level of [3H]UCB-J binding and the concentration of SV2A mRNA are strongly correlated in each patient and reduction is noted in all neuronal subtypes.

Regarding AD, the level of [3H]UCB-J binding in postmortem cortical tissue from patients with AD demonstrated that the binding was significantly lower only in the middle frontal gyrus in AD compared to matched controls. No differences were observed in the parietal, temporal, or occipital cortex. The binding levels in the frontal cortex in the AD cohort displayed large variability between subjects. Surprisingly, significant differences in binding levels were only seen in non-ApoE ε4 allele carriers, which may explain the variability.

These results strongly indicate that SV2A is an important marker for neurodegeneration. Further, the change in binding reflects gene expression, and these changes occur more in some patient subpopulations. This also raise doubt to what extent SV2A is only a marker of functional and mature synapses.

21) *Bassoon is required for presynaptic homeostatic scaling and adult ocular dominance plasticity*

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Bassoon (Bsn) is a large scaffolding protein of the presynaptic cytomatrix, involved in the structural and functional organization of neurotransmitter release. Neuronal homeostatic plasticity is a mechanism by which neurons regulate their own excitability and synaptic strength to keep network activity in the physiological range independently of synaptic input. While molecular players contributing to postsynaptic homeostatic strengthening are better understood little is known about presynaptic factors. Monocular deprivation-induced ocular dominance plasticity (MD-ODP) in the primary visual cortex (V1) is an established in vivo paradigm, in which visual deprivation-driven homeostatic network reconfiguration has been shown. Here, we explore the role of Bsn in the MD-ODP-induced functional reconfiguration of adult neocortical networks in vivo and in vitro. We subjected both juvenile and adult Bsn KO mice to MD and visualized mouse V1-activity using intrinsic signal optical imaging. We observed an absence of MD-ODP in V1 of adult constitutive Bsn KO mice, whereas juveniles were unaffected.
Importantly, experiments in a conditional model showed that selective removal of Bsn from cortical excitatory synapses also prevented adult OD-plasticity. In vitro experiments showed that Bsn deletion leads to a defect in homeostatic adaptation of glutamate release due to an aberrant regulation of synaptic vesicle (SV) recycling. Homeostatic rearrangements of postsynaptic glutamate receptors were unaffected. Finally, changes in the phosphorylation of synapsin, the main regulator of SV clustering, were evident in V1 of controls but not Bsn KOs upon MD, confirming the importance of the Bsn-dependent modulation of SV pools during MD-ODP. Together, our results establish homeostatic scaling of neurotransmitter release as an important mechanism contributing to activity-dependent modulation of cortical circuit plasticity and Bassoon as an indispensable molecular player in this process.

22) RIM and MUNC13 synergistically control neuropeptide secretion

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Neurons communicate by releasing neurotransmitters from synaptic vesicles (SVs) and neuropeptides from dense-core vesicles (DCVs). Neuropeptides specifically regulate long-term processes such as circadian rhythm, metabolism and emotions. The presynaptic active zone proteins RIM and MUNC13 are essential for both SV and DCV secretion. It is, however, unknown whether RIM and MUNC13 fulfill independent functions, which protein domains couple DCVs to release sites, and how their functions differ between SV and DCV release. Here, we show that the C2B and C2C interactions of RIM and MUNC13 control neuropeptide secretion. Using neuropeptide secretion assays with single-vesicle resolution on MUNC13/RIM quadruple null mutant neurons, we show that MUNC13 and the RIM N-terminus are both essential for DCV exocytosis. We identify the RIM N-terminus as a regulator of MUNC13 levels, which acts by preventing MUNC13 degradation. In contrast to SV exocytosis, the PIP2-binding C2B domains of RIM and MUNC13 are functionally redundant for neuropeptide secretion, whereas the lipid-binding MUNC13 C2C domain is essential. Together, these results show that RIM and MUNC13 work synergistically to control neuropeptide secretion through their C2 interactions. Our findings reveal mechanistic differences between SV and DCV exocytosis that shed new light on how the two secretory pathways diverged during evolution.

23) Role of parvalbumin-positive and somatostatin-positive interneurons in the development of prefrontal networks

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The prefrontal cortex (PFC) is considered as the hub of cognitive processing necessary for decision-making, working memory and attention. Prefrontal gamma oscillations are commonly used as proxy of neuronal processes during these tasks. According to the principle of cortical hierarchy, gamma emergence in the PFC is delayed when compared to other brain areas. In mice a prominent increase in magnitude and frequency occurs between the second and fourth postnatal week. Parvalbumin-positive (PV+) interneurons control gamma generation. The PV expression increase and the maturation of firing properties share a similar developmental time-course as the gamma oscillations. In contrast, the expression of somatostatin in Somatostatin-positive (SOM+) interneurons in the PFC remains stable with age. Here, we address the question, how PV+ interneurons are integrated in prefrontal microcircuits and if the resulting network changes contribute to the emergence of gamma oscillations. For this, we simultaneously recorded local field potential and single unit activity while optogenetically manipulating PV+ or SOM+ interneurons in the PFC of mice between postnatal week 3 and 9. To enable ontogenetic manipulations of PV+ or SOM+ interneurons, PV-Cre or SOM-Cre mice were injected with a virus containing a Cre-dependent version of Channelrhodopsin 2. We show that the ability of PV+ interneurons to exert inhibition on prefrontal activity increases with age, whereas SOM+ interneurons show adult-like activity already at the beginning of the third postnatal week. The results suggest that PV+ interneurons are embedded in prefrontal ensembles with a delay, which allows the network to generate higher gamma oscillations only from fourth postnatal week on.

24) *Synaptic phenotypes of SNAREopathy mutations characterized in mouse and human iPSC-derived neurons*

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SNAREopathies constitute a group of devastating neurodevelopmental disorders caused by mutations in the fusion machinery, which consists of the neuronal SNAREs and essential regulatory proteins that together orchestra fusion of synaptic vesicles with the plasma membrane. Core symptoms include epilepsy, intellectual disability and movement disorders, with variable clinical manifestations. In a recently published paper we found that SNAP25 disease mutations, V48F, and D166Y in the Synaptotagmin-1 binding interface, and SNARE-bundle mutation I67N, change the energy landscape for synaptic exocytosis due to aberrant SNARE interactions when virally expressed in Snap25 null mouse neurons. The interface mutations are characterized by both gain- and loss-of-function features: disinhibiting spontaneous release, enhancing binding to SNARE-partners and impairing forward priming. In contrast, bundle mutation I67N led to a higher apparent fusion barrier, thus inhibiting both spontaneous and evoked exocytosis. Studying these mutations in more patient-relevant disease models is an important next step to understand the diversity of symptoms and severities in affected humans and to design rational intervention strategies. Therefore, we generated human neurons by lentiviral NGN2-expression in CRISPR/Cas9-engineered iPSCs carrying interface mutation V48F and bundle mutation I67N. In the iPSC-derived neurons we could detect previously described changes in synaptic transmission, including increased rate of spontaneous release in the V48F mutants and reduced evoked and spontaneous release in I67N expressing neurons. In addition, we could show that the I67N mutation led to dramatic changes in short-term plasticity, which could be restored by treatment with the clinically approved 4-aminopyridine. Furthermore, we validated the V48F phenotype using patient-derived iPSCs. Finally, proteome analysis revealed dysregulation of proteins related to synapse function in the mutated human neurons.

25) *Novel Role of Endocytic Kinase AAK1 in Neuronal Autophagosome Dynamics*

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In neurons, cellular processes such as clathrin-mediated endocytosis (CME) and autophagy are crucial for maintaining cellular homeostasis and function. Clathrin-mediated endocytosis facilitates the internalization of extracellular molecules and regulates synaptic vesicle recycling during neurotransmission, while autophagy orchestrates the degradation and recycling of intracellular components, including damaged organelles and protein aggregates. The recruitment of clathrin for CME is facilitated by its major adaptor protein complex 2 (AP-2) and regulated by adaptor-associated kinase-1 (AAK1). Despite the various roles of AAK1 in cellular signaling, the consequences of AAK1 deletion for brain function remain unexplored. Here, we investigate the role of AAK1 in the brain using two novel mouse models: constitutive AAK1 knockout (KO) and excitatory neuron-confined (cKO) lines. Behavioral assessments, live-imaging electron microscopy, proteomic, and phosphoproteomic approaches reveal that AAK1 KO mice display hyperactivity, reduced body weight, and cognitive and motor deficits, but surprisingly, normal neuronal survival. AAK1 levels are highest in the cortex, and its deletion leads to the accumulation of synaptic autophagosomes in this brain region, accompanied by a concomitant reduction in synaptic vesicle number. To identify the precise cellular and molecular mechanisms of autophagosome accumulation in AAK1 KO neurons, we performed proteomic and phosphoproteomic analyses of isolated cortical synaptosomes. Deregulated autophagy/mitophagy proteins and trafficking proteins were among AAK1 substrates. Interestingly, bulk autophagy levels remained unchanged, suggesting that AAK1 might specifically contribute to autophagosome trafficking through phosphorylation of its novel brain substrates. Surprisingly, the phenotype of full-body AAK1 KO mice was fully mimicked by the phenotype of cKO mice, where AAK1 deletion in neurons was selectively driven by the CamKIIα promoter.

26) *Unveiling Synaptic Aging: A Proteomic exploration of physiological aging and the role of post-synaptic instability*

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The loss of functional synapses has been well described as a potential hallmark of brain aging. Keeping the synapse in focus, this project aims to take a step further in understanding how the process of aging can be defined as a 'synaptopathy'. By understanding the physiological aging process and extending the focus especially on the post-synaptic instability causing the loss of functional synapses, an attempt to explore the aging synapse is made.
To characterize the changes occurring through the course of physiological aging, the first step was to create a proteomic profile of a wild-type aging brain with the help of mass-spectrometry and to validate the targets. Comparisons were drawn in the synaptic fractions obtained on sub-cellular fractionation of young and old wild type adult mice ranging from 4 months to 22 months of age. To understand the impact of a structurally unstable synapse and perhaps its accelerated effect on the concept of synaptic aging, further parallels were drawn using the age-matched Shank3exΔ11(-/-) mouse model, where the post-synaptic master scaffolding protein Shank3 is functionally insufficient.
Analysis of the proteomics results pointed towards a variety of biological processes being altered upon the course of aging, possibly anticipating the loss of synaptic functions. Furthermore, synaptic count analysis to determine the synapses remaining despite the process of aging performed using the wild-type and the Shank3exΔ11(-/-) animals revealed brain-region dependent patterns. On validation of the well-known markers of aging as well as the generated targets from the proteomics analysis, a necessary contribution of the post-synaptic instability was also explored- providing evidence to the belief of aging being dependent on the availability, but additionally the structural integrity of synapses.

27) Neuronal development requires UFMylation

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Neurodevelopmental disorders (NDDs) have a highly heterogeneous etiology but share dysfunctional molecular pathways whose characterization is necessary for therapeutic development. Recently, NDDs have been linked to defects in UFMylation, a post-translational protein modification that involves the covalent and reversible attachment of UFM1 to targets via the orchestrated action of specific enzymes. Interestingly, mutations in the human Ufm1 gene or in genes encoding UFM1-conjugating enzymes have been associated with NDDs. These mutations all lead to reduced levels of UFMylated proteins in the brain, providing striking evidence that UFM1-conjugation plays a critical role in brain development and function. However, the functional role of UFMylation in the brain remains largely unknown. To increase our understanding of brain UFMylation and its role in NDD, we examined the brains and neurons of UFM1 knock-out mice, and we found that depletion of UFM1 causes cortical neurodevelopmental abnormalities. Using the immunolabeling and electrophysiological recordings in autaptic hippocampal neuron cultures, we discovered that UFM1-loss results in reduced neurite complexity, reduced synapse number and corresponding changes in synaptic transmission. At the molecular level, these phenotypic changes are paralleled by reduced neuronal protein translation. Our data show that UFMylation co-controls neuronal development and differentiation, likely by guaranteeing proper protein synthesis.

28) *Protein Transport from Synapse-to-Nucleus is essential for Long-Lasting Activation of CREB following Induction of Synaptic Plasticity*

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Neuronal plasticity is a tightly regulated cellular mechanism that is essential for the formation of memory. A complex interplay of signaling pathways and molecular players orchestrate the activity of the transcription factor CREB, a central driver of neuronal plasticity, during distinct phases and on different timescales. While the induction of long-term potentiation causes an initial upregulation of CREB activity through electrochemical signaling, we show that the maintenance of its activity critically depends on another type of signaling that is mediated by the messenger protein Jacob, which translocates from dendritic spines to the nucleus via the molecular motor dynein.
The translocation process is initiated by a sequence of molecular processes in dendritic spines following synaptic activity, involving interaction of Jacob with the Ca2+-sensor caldendrin and the F-actin binding protein cortactin, to redistribute Jacob from the PSD to the spine base. This position is particularly favorable, as we show that Jacob subsequently uses spine-invading microtubules to leave the spine head and translocate to the nucleus.
As hub for protein interaction, Jacob forms a signalosome with other proteins. We show that a trimeric complex of Jacob with importin-α and –β constitutes the core of the signalosome. It couples the complex to dynein and grants a high degree of stability which is critical to protect it from disintegration during the translocation process. Importantly, the signalosome also contains the kinase (p)ERK which, once the complex reaches the nucleus, maintains the phosphorylation and activity of CREB to drive neuronal plasticity.
Intriguingly, only those spines that contain the whole molecular machinery for the formation of the signalosome may participate in this form of signaling. Therefore, we predict the existence of a distinct set of spines that play an instrumental role in synapse-to-nucleus signaling and subsequent gene expression.

29) *Biogenesis of neuronal signaling amphisomes at presynaptic terminals following high-frequency stimulation*

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Amphisomes are hybrid organelles that result from the fusion of late endosomes with autophagosomes at presynaptic terminals. In a previous study we showed that signaling amphisomes in neurons are not only endowed with a degradative role by engulfing cargo and actively transport it to the soma to form autolysosomes but they also serve signaling functions by incorporating active TrkB receptors and allowing local TrkB signaling at presynaptic boutons during their retrograde transport (Andres-Alonso et al.,2019). Here we investigated mechanisms governing the biogenesis of signaling amphisomes and we propose that signaling amphisomes are formed at presynaptic terminals of mature neurons following high-frequency stimulation. In line with this, we discovered that high-frequently stimulation of primary hippocampal neurons leads to rapid autophagy induction at presynaptic boutons that correlates with a significant increase of signaling TrkB positive-amphisomes in axons. Moreover, we found that high-frequency stimulation induces formation of bulk endosomes at the terminals which we labeled with a high-molecular weight Dextran, and our data indicates that activity dependent bulk-endocytosis might play a role as a membrane source for amphisome formation at the presynapse. What could be the mechanisms leading to amphisome biogenesis at boutons? We propose that activation of the energy-sensing enzyme AMPK is essential for the initiation of the canonical autophagy pathway at boutons. In agreement with this, interventions aimed to prevent AMPK activation following high synaptic activation resulted in a significant reduction in the initiation of autophagy at boutons and it prevented the biogenesis of amphisomes following high-frequency stimulation. In summary, our study indicates that amphisome biogenesis occurs following high-frequency stimulation and depends on both the activation of the canonical autophagy pathway through AMPK activation and bulk endosomes for their formation.

30) Effects of Perturbed Sumoylation, Neddylation, and Ufmylation on Synaptic Transmission

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Post-translational modifications of proteins by Ubiquitin-like modifiers (Ubls) regulate a wide range of cellular processes. Indeed, increasing evidence links Ubls to neuronal development, but their role at synapses remains enigmatic. To address this issue, we examined the role of three essential Ubls, SUMO2, NEDD8, and UFM1, in synaptic transmission. Using autaptic hippocampal cultures from corresponding conditional knock-out mouse lines, we combined imaging approaches and electrophysiological recordings to systematically characterize the morphology and synaptic properties of excitatory glutamatergic neurons lacking SUMO2, NEDD8, or UFM1. SUMO2 deficient neurons showed reduced dendrite complexity and synapse numbers, along with a decrease in evoked and spontaneous synaptic transmission, and consequently reduced synaptic short-term depression. In contrast, NEDD8-deficient neurons showed increased vesicular release probability and consequently increased synaptic short-term depression, with largely unaltered morphology. Finally, UFM1-deficientneurons showed severely reduced dendrite complexity and synapse numbers, along with strong decreases in evoked and spontaneous synaptic transmission, while synaptic short-term depression was not changed. Altogether, our data indicate that SUMO2, NEDD8, and UFM1 each have specific ‘synaptic signatures’ and regulate the development and function of neurons via largely distinct mechanisms.

31) *Timing is of the essence: desynchronized synaptic transmission as novel disease mechanism in SYT1-associated disorder*

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Synaptotagmin-1 (Syt1) is a presynaptic calcium sensor that triggers action potential-evoked synchronous neurotransmitter release, while suppressing asynchronous and spontaneous release. We identified a de novo missense mutation (P401L) in SYT1 carried by a patient with developmental delay and autistic symptoms. Expressing the orthologous mouse mutant (P400L) in cultured Syt1 null neurons revealed a reduction in dendrite outgrowth and number of synapses, which was dependent on synaptic input. Patch-clamp recordings showed that spontaneous neurotransmitter release per synapse was increased more than 500% in Syt1PL-expressing neurons, even beyond the increased rates in Syt1 KO neurons. Furthermore, action potential-evoked asynchronous release was increased more than 100%, while synchronous release was unchanged. Over-expression of Syt1PL in wild type neurons yielded similar results. Our findings show that Syt1PL desynchronizes neurotransmission by reducing the suppression spontaneous and asynchronous release. Neurons respond to this by shortening their dendrites, possibly to counteract the increase in release. Syt1PL acts in a dominant-negative manner supporting a causative role for the mutation in the heterozygous patient. We propose that the substitution of a rigid proline to a more flexible leucine at the bottom of the C2B domain impairs clamping of release by interfering with Syt1's primary interface with the SNARE complex. This is a novel cellular phenotype, distinct from what was previously found for other SYT1 disease variants, and points to a role for spontaneous and asynchronous release in SYT1-associated neurodevelopmental disorder.

32) A fully titratable in vitro model for E/I-balance using human neurons converges to stable network oscillations

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The balance between excitation and inhibition (E/I-balance) in synaptic inputs to a neuron and in neural network is important for correct brain development and function. To gain a better understanding of E/I-balance and its role in pathophysiology, induced pluripotent stem-cells (iPSCs) can be used to generate patient-own neurons in vitro. Here, we compare multiple GABAergic differentiation protocols and use induced glutamatergic and GABAergic neurons to study human-derived E/I-networks. IPSC-derived GABAergic differentiation was performed using multiple protocols, and their performance was studied using immunocytochemistry, mass-spectrometry proteomics, and electrophysiology. Networks oscillations of E/I-networks containing induced glutamatergic and GABAergic neurons were measured using calcium-imaging techniques, and a computation model of the network was built to interpret the findings. Our findings suggest that our novel induction protocol allows for more reliable GABAergic differentiation then established protocols. These induced GABAergic neurons integrate fully in E/I-networks with induced excitatory neurons, and networks are fully titratable. Networks retain cellular ratios over prolonged culture, and synaptic projections scale with cellular ratios. Functionally, we found that irrespective of E/I-ratio, networks oscillations are remarkably stable. Using in-silico methods, we propose a mechanism that could be underlying this convergent property.

33) *Investigating the role of CaMKII in hippocampal synaptic plasticity using all-optical approaches*

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Calcium-calmodulin-dependent protein kinase II (CaMKII) is an important memory molecule that transforms transient synaptic activity events into long-lasting synaptic plasticity through its autophosphorylation feature. Importantly, dysregulation of CaMKII is increasingly implicated in synaptopathy, possibly through maladaptations in synaptic plasticity. We are interested in the role of CaMKII in synaptic plasticity. Specifically, it is controversial whether CaMKII is essential to induce and/or maintain plasticity. We took advantage of optogenetic tools to investigate the role of CaMKII in synaptic plasticity, inducing plasticity and manipulating relevant signaling pathways simultaneously. We induced spike-timing-dependent plasticity at Schaffer collateral synapses in hippocampus by the optogenetic stimulation of two neuronal populations expressing spectrally distinct channelrhodopsins. The all-optical method induced time-dependent long-term potentiation (tLTP), increasing synaptic strength both acutely (minutes) and more interestingly, chronically (days). When we optically inhibited CaMKIIα activity while inducing tLTP, a full blockade of acute tLTP was observed. Surprisingly, 3 days later, stimulated neurons received stronger inputs than their neighbors, a delayed potentiation that appears to be independent of CaMKIIα activity. Coincidently, the expression of an immediate early gene Fos also didn't depend on CaMKIIα. Conversely, direct optical activation of CaMKIIα was sufficient to induce acute functional & structural LTP, and ultrastructural changes. However, this CaMKIIα activation-induced LTP returned to baseline after 2 days. Together, our work suggests that activity-dependent potentiation of synaptic inputs has two phases: CaMKIIα is necessary & sufficient for the induction of early LTP. A second, CaMKIIα independent mechanism, possibly through the persistent activity of protein kinase Mζ, is responsible for the selective strengthening of inputs days later.

34) *Regulation of cortical synaptic development by the glutamate receptor delta-2*

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Ionotropic glutamate receptors (iGluRs) of the delta subfamily (namely GluD1 and GluD2) are atypical receptors. They do not bind glutamate and they mostly act as synaptic organizers through non-ionotropic signaling. GluDs are postsynaptic receptors that mediate trans-synaptic interactions with presynaptic neurexins and secreted cerebellins (Cblns). So far, most studies on GluD receptors have focused on the role of GluD2 in the cerebellum, where it is highly expressed. In the cerebellum, GluD2 is required for the formation, maintenance and plasticity of excitatory synapses between parallel fibers and Purkinje cells, and mutations in GRID2, the gene encoding GluD2, cause spino-cerebellar ataxia. Recently, single-cell transcriptomics and immunohistochemistry studies have revealed that GluD2 is also expressed in the neocortex. Accordingly, GRID2 mutations have been implicated in autism and schizophrenia but the role of GluD2 outside of the cerebellum has never been investigated.
In this study, we aimed at understanding the function of GluD2 in cortical synaptic development. We used in utero electroporation to knock down endogenous GluD2 expression in layer 2/3 cortical pyramidal neurons (CPNs) and we analyzed synapses in juvenile (postnatal day 21) mice. We found that GluD2-deficient neurons displayed a lower density of dendritic spines but the remaining spines had larger heads than in control neurons. These alterations were associated with changes in excitatory transmission. In line with these results, we found that GluD2 localizes in dendritic spines. Using an in vivo gene replacement strategy, we show that the regulation of dendritic spine formation by GluD2 requires the interaction with Cblns but not ion flux through the pore or agonist binding, indicating that GluD2 operates through trans-synaptic non-ionotropic signaling. Together, our results highlight a major role for GluD2 outside of the cerebellum in the development and the connectivity of cortical circuits.

35) *DUOX2 acts as a coincidence detector to activate the NAADP – IL-17 axis in T cells*

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NAADP is a potent Ca2+ releasing second messenger with an important role in T cell activation. After stimulation, NAADP is rapidly formed, contributing to the first Ca2+ signals in T cells. This impacts T cell effector functions, making the NAADP signaling pathway an attractive target for pharmacologic interventions. The therapeutic potential of modulating NAADP signaling was demonstrated in a rat model of multiple sclerosis, further highlighting the importance of identifying the key components of this signaling pathway. After our recent discovery that DUOX2 synthesized NAADP during T cell activation, we now followed up by investigating the molecular mechanisms controlling NAADP synthesis in T cells. In enzyme assays, we discovered a regulation by nanomolar changes in the free Ca2+ concentration and phosphorylation by PKA Cβ2 and PKCθ. We confirmed their role in primary T cells obtained from knockout mice using high-resolution Ca2+ imaging and stimulation with αCD3/ αCD28 antibody-coated beads. Additionally, we show priming of T cells by TCR independent Ca2+ microdomains resulting from store-operated Ca2+ entry and purinergic signaling by analyzing tempo-spatial correlations of TCR-independent and -dependent Ca2+ microdomains in new and previously published datasets. Next, we used Epac-based FRET-sensors to demonstrate an autocrine signaling axis from pannexin1-mediated ATP release and extracellular metabolization by ecto-nucleotidases including CD73 that sustains basal A2AR-signaling and cAMP levels in unstimulated T cells and is required for the activation of DUOX2 by PKA Cβ2. Finally, we show that both the A2AR/ PKA Cβ2 and the TCR/ PKCθ pathway for activation of the DUOX2/NAADP/Ca2+ signaling axis can be targeted using pharmacological compounds that have already demonstrated safety in clinical trials. These compounds synergistically target the development of IL-17+ T cells, uncovering a novel approach to selectively target NAADP-signaling in autoreactive T cells.

36) Neurobeachin regulates receptor downscaling at GABAergic inhibitory synapses in a protein kinase A-dependent manner

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GABAergic synapses critically modulate neuronal excitability, and plastic changes in inhibitory synaptic strength require reversible interactions between GABAA receptors (GABAARs) and their postsynaptic anchor gephyrin. Inhibitory long-term potentiation (LTP) depends on the postsynaptic recruitment of gephyrin and GABAARs, whereas the neurotransmitter GABA causes the synaptic removal of GABAARs. However, the mechanisms and players underlying plastic adaptation of synaptic strength are poorly understood. Here we show that neurobeachin (Nbea), an autism risk factor and protein kinase A (PKA) anchor protein involved in receptor trafficking, is a component of inhibitory synapses and regulates the downregulation of inhibitory synaptic transmission. We found that the recruitment of Nbea to GABAergic synapses is activity-dependent and that Nbea interacts with postsynaptic gephyrin. In heterozygous neurons lacking one Nbea allele, PKA inhibition or overexpression of a PKA binding-deficient mutant impaired synaptic GABAAR removal. Our data suggest a mechanism by which Nbea mediates PKA anchoring at inhibitory postsynaptic sites to downregulate GABAergic transmission. They emphasize the importance of kinase positioning in the regulation of synaptic strength.

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