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MEMBRANES AND THE CYTOSKELETON IN HEALTH AND NEURODEGENERATIVE DISEASE

10th Westerberger Herbsttagung on the Perspectives Of Molecular Neurobiology

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Introduction

We are very pleased to welcome you to the 10th Westerberger Herbsttagung on the Perspectives of Molecular Biology - "Membranes and the Cytoskeleton in Health and Neurodegenerative Disease". The meeting usually takes place every two years and was launched in 2002 to mark the founding of the Department of Neurobiology at Osnabrück University. This year we are particularly pleased to be able to hold the meeting as we had to cancel our last one scheduled for 2020 due to the COVID19 precautions. In addition, this meeting also marks an anniversary and coincides with 20 years of the Department of Neurobiology in Osnabrück.

The aim of the Westerberger Herbsttagung is to bring together a group of excellent researchers on a specific topic in a friendly and communicative atmosphere. Topics of the past Westerberger Herbsttagungen have been “Cell and Brain Imaging”, “Neural Communication and Dynamics” or “Psychological and Biological Aspects of Explicit and Implicit Memory”. Booklets of the past Westerberger Herbsttagungen are available on the Department’s website (https://www.neurobiologie.uni-osnabrueck.de). This year we have somehow returned to our roots as a cytoskeleton group and are pleased that we have once again managed to bring together a group of excellent researchers from Europe to discuss this year's main theme, "Membranes and the Cytoskeleton in Health and Neurodegenerative Disease".

As every time, the Westerberger Herbsttagung is also an opportunity for the Department's early career researchers to present their science to experts from the field. Therefore, I am happy that we will also have four progress talks and five posters on selected projects that are currently carried out in the Department.

The Westerberger Herbsttagung is organized by the whole Department of Neurobiology and I would like to thank everyone for their input on the various aspects of organizing such an event. This includes preparation of the announcement, the planning of the program and the selection of the buffet. We hope that we will be able to provide a pleasant atmosphere for fruitful discussions.

Finally, I would also like to thank the sponsors of this meeting, in particular the EU consortium TubInTrain, the SFB 944, Nikon, which traditionally supports our Speaker's Dinner, as well as Sarstedt, Diagonal and Macherey-Nagel, who regularly support the Westerberger Herbsttagung. We also thank the Center for Cellular Nanoanalytics Osnabrück (CellNanOs) and the University for providing rooms and infrastructure.

We look forward to an exciting conference.

Roland Brandt
(for the organizing committee)

10th Westerberger Herbsttagung zu den Perspektiven der Molekularen Neurobiologie

MEMBRANES AND THE CYTOSKELETON IN HEALTH AND NEURODEGENERATIVE DISEASES

Date: Thursday, September 29, 2022

Place: Center for Cellular Nanoanalytics (CellNanOs), Barbarastrasse 11, room 38/201, Osnabrück

Organizational Committee:

Nanci Abreu, Simone Attanasio, Lidia Bakota, Roland Brandt, Anna-Carina Söhnel, Ahmed Soliman, Nataliya Trushina, Kerli Tulva.

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Program

9:00 Introduction: Roland Brandt (UOS)

9:05 Welcome address by Kai-Uwe Kühnberger, Vice President for Research and Promotion of Young Talent at Osnabrück University

Session I: (Chair: L. Bakota)

9:15 Lecture 1: Nicolas Vitale, PhD (Centre National de la Recherche Scientifique, Université de Strasbourg, France):

"Phosphatidic acid: a key lipid in normal and pathological neuronal function"

10:00 Lecture 2: Tiago Gil Oliveira, MD, PhD (Life and Health Sciences Research Institute, School of Medicine, University of Minho, Braga, Portugal)

"The role of the phospholipase D pathway in hippocampal functioning and Alzheimer's disease"

10:45 Coffee Break

11:15 Lecture 3: Hilal A. Lashuel, PhD (Laboratory of Chemical Biology of Neurodegeneration, Brain Mind Institute, Swiss Federal Institute of Technology Lausanne, Switzerland):

"Rethinking protein aggregation and drug discovery in neurodegenerative diseases"

12:00 Progress Talk 1: Nataliya Trushina (Neurobiologie, UOS):

"Mass spectrometry analysis of Alzheimer's disease-like cell model reveals changes in factors responsible for cytoskeleton dynamics"

12:15 Progress Talk 2: Kerli Tulva (Neurobiologie, UOS):

"The axon initial segment is altered in a tau dependent manner in various brain regions of mice"

12:30 Progress Talk 3: Simone Attanasio (Neurobiologie, UOS):

"Dissecting the effect of extracellular tau on tau in cells: A new cellular model for understanding tauopathies"

12:45 Progress Talk 4: Ahmed Soliman (Neurobiologie, UOS):

"Investigation of the dynamic interplay between the aggregation prone Tau-ΔK280 and microtubules"

13:00 Lunch, Coffee and Poster session

Session II: (Chair: R. Brandt)

15:15 Lecture 4: Anne Straube, PhD (Warwick Medical School, University of Warwick, Coventry, United Kingdom):

"Regulation and force generation of neuronal transporter KIF1C"

16:00 Lecture 5: Stefan Kins, PhD (TU Kaiserslautern, Kaiserslautern, Germany)

"The Amyloid precursor protein and its adaptor Fe65/L1 regulate actin cytoskeleton dynamics in growth cones and spines"

16:45 Concluding remarks: R. Brandt

17:00 End of Meeting

Keynote Lectures

Lecture 01

Phosphatidic acid: a key lipid in normal and pathological neuronal function

Nicolas Vitale, PhD

Centre National de la Recherche Scientifique, Université de Strasbourg, Institut des Neurosciences Cellulaires et Intégratives, F-67000 Strasbourg, France

Lipids have been recently suspected to contribute to optimal neuronal function, but the precise mechanisms remain elusive. Using a combination of pharmacological and molecular approaches associated with genetic mouse models, we have recently shown that phosphatidic acid (PA) produced by PLD1 regulates the various steps of neurosecretion using neuroendocrine chromaffin cells as a model. Interestingly these observations revealed that mono-unsaturated PA control the number of exocytotic events most likely by contributing to granule recruitment/docking, whereas poly-unsaturated PA regulate fusion pore stability and expansion. We also found that PA modulates secretory granule biogenesis, transport, and recycling, revealing a very complex regulation of the entire life cycle of secretory vesicles by PA. Altogether, this work opens a novel insight into the different roles in a given cellular function that subspecies of the same phospholipid may play based on their fatty acyl chain composition and highlights one of the possible functions of polyunsaturated fatty acids during neurosecretion. Finally, we will present evidences that alteration of PA dynamics may contribute to neuronal pathology.

Lecture 02

The role of the phospholipase D pathway in hippocampal functioning and Alzheimer's disease

Tiago Gil Oliveira, MD/PhD

Life and Health Sciences Research Institute (ICVS), ICVS/3B's, School of Medicine, Campus de Gualtar, University of Minho, 4710-057 Braga, Portugal

Lipids are a major constituent of the brain and have been implicated as key mediators of Alzheimer's disease (AD) pathogenesis. Recent advances in mass-spectrometry lipidomics showed that specific lipid signaling pathways are differentially regulated in particular physiological and disease states. The hippocampus is a brain structure relevant for learning and memory and is impaired in AD. In an unbiased lipidomic study, we compared the lipid composition along its longitudinal axis, and among many relevant differential lipid changes, we showed a phosphatidic acid gradient, with higher levels in the dorsal hippocampus. At the pathological level, we had also shown that amyloid beta increased the activity of the lipid-modulating enzyme, phospholipase D (PLD). In mammals there are two main canonical PLDs, PLD1 and PLD2, and both convert phosphatidylcholine into phosphatidic acid. Interestingly, while the ablation of PLD2 in an amyloidogenesis model had a protective effect at the synaptic and behavioral levels, the ablation of PLD1 had a deleterious effect by impairing the functioning of the hippocampus along its longitudinal axis. Therefore, in order to understand the mechanism of protection conferred by PLD2 ablation in AD models, we are currently characterizing the molecular regional hippocampal changes along its longitudinal axis.

Lecture 03

Rethinking protein aggregation and drug discovery in neurodegenerative diseases

Hilal A. Lashuel, PhD

Swiss Federal Institute of Technology Lausanne (EPFL) and CSO of ND BioSciences, Lausanne, Switzerland

More than a century has passed since pathological protein aggregates were first identified as defining hallmarks for several neurodegenerative diseases (NDDs), including Alzheimer's disease, Parkinson's disease. Over the years, converging evidence from human genetics, neuropathological studies, and animal models pointed to a central role for protein aggregation in the initiation and progression of NDDs. This has led to the pursuit of different small molecule and antibody-based therapeutic strategies to prevent protein aggregation or stop pathology from spreading in the brain. Yet, we still do not have effective therapies to treat or slow the progression of these devastating diseases or diagnostics for early detection and monitoring disease progression.

In this lecture, I will reflect on some of the reasons underlying the drug development failures in NDDs, including the failure to embrace the neuropathological complexity and clinical heterogeneity of NDDs. Using Parkinson's disease as a case study and recent findings from our group, I will demonstrate how new advances in chemical biology, imaging, protein structure amplification, and omics technologies have enabled us to deconstruct and reconstruct the complexity of brain pathologies and disease mechanisms in NDDs. These advances have provided novel insights that challenge the traditional views and hypotheses on the role of protein aggregation in NDDs. I will close by making the case that effective treatments of PD and other NDDs will require personalized combination therapies that account for the clinical heterogeneity of the disease and the complexity of the molecular mechanisms driving neurodegeneration and disease progression.

Lecture 04

Regulation and force generation of neuronal transporter KIF1C

Anne Straube, PhD

Warwick Medical School, University of Warwick, Coventry CV4 7AL, United Kingdom

Intracellular transport is essential for neuronal function and survival. The most effective plus end-directed neuronal transporter is the kinesin-3 KIF1C, which transports large secretory vesicles and endosomes. Mutations in KIF1C cause hereditary spastic paraplegia and cerebellar dysfunction in human patients. I will discuss the single molecule mechanics and mechanisms of autoinhibition and release that control the activity of KIF1C.

In contrast to other kinesin-3s, KIF1C is a stable dimer and a processive, plus-end directed motor. The microtubule binding surface of KIF1C motor domain interacts with its stalk. These autoinhibitory interactions are released upon binding of protein tyrosine phosphatase PTPN21 or cargo adapter Hook3 to the KIF1C stalk. This autoinhibition release mechanism enables cargo-activated transport. In primary hippocampal neurons, the FERM domain of PTPN21 stimulates dense core vesicle transport.

KIF1C molecules can processively step against the load of an optical trap and reach average stall forces of 3.7pN. Compared to kinesin-1, KIF1C has a higher propensity to slip backwards under load, which results in a lower maximal single molecule force. However, KIF1C remains attached to the microtubule while slipping backwards and re-engages quickly consistent with its super-processivity. Two pathogenic mutations P176L and R169W that cause hereditary spastic paraplegia in humans maintain fast, processive single molecule motility in vitro, but with decreased run length and slightly increased unloaded velocity compared to the wildtype motor. Under load in an optical trap, force generation by these mutants is severely reduced. In cells, the same mutants are impaired in producing sufficient force to efficiently relocate organelles.

Our results show how its mechanics supports KIF1C's role as an intracellular transporter and explain how pathogenic mutations at the microtubule-binding interface of KIF1C impair the cellular function of these long-distance transporters and result in neuronal disease.

Lecture 05

The Amyloid precursor protein and its adaptor Fe65/L1 regulate actin cytoskeleton dynamics in growth cones and spines

Stefan Kins, Dr. rer. nat.

Technische Universität Kaiserslautern, Erwin-Schrödinger-Straße, Gebäude 13, Kaiserslautern, Germany

The amyloid precursor protein (APP) gets proteolytically cleaved into different fragments, including the amyloid-β peptide, which aggregates in the brain of Alzheimer's patients. Thus, it plays a key role in the pathogenesis of Alzheimer's disease. The proteolytic processing of APP is very well studied, whereas the physiological function of APP and its cleavage products is only poorly understood. Important insights into the in vivo functions of the APP gene family have been gained by analyzing engineered mouse mutants, lacking APP, which exhibit defects in neuronal migration, dendritic branching and synaptic plasticity. Interestingly, many of the APP-KO phenotypes resemble those of mice lacking the intracellular APP-binding partner Fe65. Here, we provide strong evidence that both APP and Fe65 are critically involved in regulating actin dynamics in growth cones and spines, as demonstrated by live cell imaging and FRAP analyses. Our studies suggest that APP likely recruits Fe65 and various actin regulators to subcellular sites necessary for de novo actin filament formation. This important function in regulating the actin cytoskeleton may also explains the diverse phenotypes observed in APP and Fe65-KO animal models.

Progress Talks

Progress Talk 01

Mass-spectrometry analysis of Alzheimer's disease-like cell model reveals changes in factors responsible for cytoskeleton dynamics

Nataliya I. Trushina

Department of Neurobiology, Osnabrück University, Osnabrück, Germany

The microtubule-associated protein tau is important for microtubule cytoskeleton regulation and function. Dysregulation, in particular, hyperphosphorylation, of tau is associated with developing a group of neurodegenerative diseases called tauopathies, including Alzheimer's disease.
As a model for tau hyperphosphorylation, we used a phosphomimicking human tau construct in which ten major phosphorylation sites, previously identified in patients with Alzheimer's disease, were mutated to glutamate residues. Using mass spectrometry, we analyzed changes in the proteome of model neurons (differentiated PC12 cells) expressing pseudohyperphosphorylated (PHP) tau compared to wildtype tau.
Functional analysis of differentially expressed proteins showed enrichment in proteins associated with the cytoskeleton. One significantly increased protein in PHP tau expressing cells was tubulin-sequestering protein stathmin 2. It is a neuronal protein that promotes microtubule instability, a feature necessary for axonal outgrowth and regeneration but can disturb normal microtubule dynamics in developed cells. Western blot analysis confirmed stathmin 2 upregulation in differentiated PHP tau expressing cells.
To correlate the proteomics data with functional changes, we further analyzed changes in microtubule dynamicity via live cell imaging technique. In axon-like processes, microtubule polymer was reduced upon expression of PHP tau. Using a recently established super-resolution imaging technique for quantitative analysis of neuronal microtubules, DNA-PAINT, we also showed a lower density of microtubules in these cells. Moreover, another marker of microtubule stability – the amount of acetylated tubulin – was also decreased in PHP tau-expressing cells.
Analysis of tubulin dynamics upon exogenous expression of stathmin 2 showed that an increase of this protein is sufficient to observe a lower amount of polymer in model neurons.
Finally, we looked at the expression of stathmin 2 in brain samples from AD patients compared to control patients, and it was significantly increased, showing that the observed changes can be translated to more complex conditions.
Thus, we showed one of the potential mechanisms via which hyperphosphorylation of tau can cause microtubule cytoskeleton structural changes and be a sign of pathology development.

Progress Talk 02

The axon initial segment is altered in a tau dependent manner in various brain regions of mice

Kerli Tulva

Department of Neurobiology, Osnabrück University, Osnabrück, Germany

The axon initial segment (AIS), located at the proximal part of the axon where the axon potentials are generated. It also contributes to the molecular identity of the axon by, for example, enabling enrichment of tau within the process. In this study the potential influence of tau is investigated on the structure, position and physiological role of AIS. AnkyrinG was used as a marker of the AIS, and laser scanning micrographs were analyzed for the length of the AIS and the distance from the cell body. AIS structure and position was first analyzed in primary hippocampal cultures which showed that AIS was longer in the neurons from TauKO mice compared to controls. After introducing full length tau or tau lacking the N-terminal domain to the primary neuronal cells, we found that the AIS length in TauKO mice was restored to the level of the controls. Next, the AIS parameters were investigated in young (3M old) and aged (1Y old) TauKO mice and the control strain where also cortex and amygdala were analysed. Interestingly, we observed a shorter AIS in the adult TauKO mice compared to control, which reached significance in all of the four brain regions at 3M of age. Electrophysiological recordings using multi-electrode array (MEA) in the hippocampal CA1 region were taken to characterize the differences in long term potentiation (LTP). These results showed that 3M and 1Y old TauKO mice had higher LTP in the stratum pyramidale. Our data shows that AIS is altered in TauKO mice and exogenously introduced tau restores the AIS structure, suggesting a critical role of tau in the organization of AIS.

Progress Talk 03

Dissecting the effect of extracellular tau on tau in cells: A new cellular model for understanding tauopathies

Simone Attanasio

Osnabrück University, Department of Neurobiology, Osnabrück, Germany;
University of Milan, Bioscience, Milan, Italy;

The microtubule associated protein Tau is necessary for the correct functioning and structure of the cytoskeleton. Tau interacts with microtubules with a fast kiss-and-hop mechanism. This interaction can be impaired by post translational modifications, phosphorylation, or gene mutations. All these factors, associated with neurodegenerative diseases like Alzheimer's disease (AD), also referred as Tauopathies, increase the propensity of monomeric Tau to form insoluble oligomers/aggregates.
Mutation at residue 280 (ΔK280del), previously reported in Frontotemporal dementia and associated with late onset AD, increases Tau aggregation rate in vitro. To investigate the effect of this deletion we used NGF-differentiated PC12 cells transfected with PAGFP-Tau441 or PAGFP-TauΔK280. The decay after photoactivation (FDAP) was followed in the axon like process. We found that the amount of Tau interacting with MTs is significantly reduced in TauΔK280 compared to Tau441.
To understand the role of aggregation in this change, we tested different conditions, exposing our model neuron to different pre-sonicated aggregates. Monomeric protein (Tau441 or ΔK280) was aggregated in the presence of Heparin and cells were exposed to aggregates 24 hours before imaging.
We found that external ΔK aggregates lead to an increased Deff and a decreased MT-Tau bound fraction only in the presence of PAGFP-TauΔK280, but not in the presence of PAGFP-Tau441. On the other side, external Tau441 aggregates didn't lead to a significant change in any condition.
Finally, we also tested the effect of monomeric ΔK280 in PAGFP-ΔK280 transfected cells. We found that the monomeric Tau is already sufficient to cause a change in the interaction between Tau and the microtubules.
Together our finding suggest that the interaction is affected by external ΔK280 fibrils or external ΔK280 monomers. In these conditions, a decreased MT-Tau bound fraction suggests a higher availability of monomeric Tau to form further oligomers/aggregates in the cellular compartment.
This effect may be due to a direct uptake of ΔK280 aggregates/monomers which may function as seeding templates in the cell. However, it could also be the result of a cellular cascade that doesn't require the toxic species uptake.

Progress Talk 04

Investigation of the dynamic interplay between the aggregation prone Tau-ΔK280 and microtubules

Ahmed Soliman

Department of Neurobiology, Osnabrück University, Osnabrück, Germany

The microtubule associated protein Tau is abundant within neurons. It regulates the microtubules (MTs) dynamics through promoting the assembly and bundling of MTs. Tau-MTs interaction changes can impact the cellular functional mechanisms that may result in neurodegenerative diseases progression, such as: Frontotemporal dementia. Those changes may occur due to a mutation in the Tau's gene as we demonstrate in this study the Tau-ΔK280 construct.
Here, we conducted a study including structural- and neuro-biological investigations of Tau-441-wt and Tau-ΔK280 interactions with MTs. On the structural biology level, we showed that both of the Tau proteins of interest can interact with MTs in in vitro tubulin polymerization assays. Then, we correlated that interaction through observing the sedimentation co-efficient of the proteins upon decorating MTs and the intra-microtubular structural alterations. On the neurobiological aspect, we transduced Tau-ΔK280 construct, with a green fluorescent tag, into primary mice dorsal root ganglia (DRG) culture. Thereafter, we performed live cell imaging to monitor the mutant Tau-MTs interaction in response to aging and drug treatment. Tau-ΔK280 construct showed a significant higher diffusion across DRG MTs in comparison to Tau-441-wt. In addition, we showed that aging of neurons can exacerbate that interaction perturbation.
Through this study, we could correlate the nature of interaction of Tau-ΔK280 and Tau-441wt with assembled MTs in cellular and cell-free systems. Last, we provided a pharmacological approach to tackle the Tau-MTs dynamic alterations detected.

Poster Abstracts

Poster 01

Methods to assess the activity of drug candidates on tau aggregation and tau microtubule dynamics

Nicolò Bisi[1,2], Christian Conze[1], Davide di Lorenzo[2], Sandrine Ongeri[2] and Roland Brandt[1]

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[2]BioCIS, CNRS, Université Paris-Saclay, Chatenay-Malabry Cedex, France

Tau proteins are among the most studied Microtubule Binding Proteins (MAPs), playing a crucial role in regulating microtubule (MT) dynamics [1]. In healthy humans, tau is mainly found in the axon of brain neurons. However, in tauopathies like Alzheimer's Disease (AD), tau undergoes hyperphosphorylation and aggregates in neurofibrillary tangles (NFTs) in the somadendritic compartment of the neuron. This finally leads to MT depolymerization and cell toxicity. Up to now, no therapeutic treatment is available to inhibit tau aggregation and prevent the associated neurodegeneration [2].
We developed methods to identify compounds able to inhibit tau aggregation in vitro and to restore the tau – MT interaction in cells. The approach is based on a full-length tau construct prone to pathological aggregation, tauΔK280. We confirmed increased aggregation of purified, recombinant tauΔK280 compared to wild-type tau in cell-free assays. By Fluorescence Decay After Photoactivation (FDAP) imaging, we demonstrated reduced binding of PAGFP-tagged tauΔK280 to microtubules in axon-like processes in model neurons as evidenced by a higher effective diffusion rate.
Our data show that the approach allows to assess the activity of novel tau aggregation inhibitors in cell free assays and their ability to restore the physiological tau-MT interaction in model neurons.

References:
[1] Arendt et al. (2016) Tau and tauopathies. Brain Res Bull. 126:238-292;
[2] Brandt R, and Bakota L (2017) Microtubule dynamics and the neurodegenerative triad of Alzheimer's disease: The hidden connection. J. Neurochem. 143:409-417

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Poster 02

Super-resolution imaging and quantitative analysis of neuronal microtubule lattice in 3D

Nataliya Trushina[1], Christian Conze[1], Michael Holtmannspötter[2,3], Niklas Laasch[1], Marina Rierola[1], Lidia Bakota[1], Jacob Piehler[2, 3], Roland Brandt[1, 2, 4]

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Microtubules (MTs) are essential for the development of neurons and maintaining their structure. MTs serve as a central system for the long-distance transport of cargo. The organisation and dynamics of neuronal MTs are influenced by various factors, and disturbance of their regulation can contribute to neurodegenerative diseases.
Imaging MT filaments in densely packed neuronal processes is challenging. However, the development of super-resolution techniques combined with the use of nanobodies offers new possibilities to visualise and quantitatively analyse neuronal MT organisation.
We implemented DNA-PAINT (Point Accumulation in Nanoscale Topography), a single-molecule localisation microscopy (SMLM) technique, that allowed us to acquire 3D arrays of the MT lattice in axons of model neurons and dendrites of primary neurons.
As a first approach, for the quantitative analysis of MTs, we used the SMLM image filament extractor (SIFNE) [1]. 2D projections of optical sections with 100 to 150 nm thickness having optimal resolution were used to infer various parameters of the MT lattice. For validation of the method, we performed MT lattice analysis upon stabilising and destabilising conditions. We found significant changes in MT organisation concerning the length of individual MTs, filament straightness, and polymer density in neuronal processes affecting the frequency of intersecting filaments. Changes in MT mass were consistent with measurements of ensemble MT dynamics.
To use the full potential of our 3D imaging strategy, we established a protocol to assess MT structure in 3D. We used “Polyphorm”, a versatile visualisation and model fitting tool [2]. The generated trace field enabled 3D data segmentation, which was further used to analyse the orientation and assign the associated filaments in the vicinity.
This new technique will be presented, offering the possibility of improved data analysis and future semi-automated processing to quantitatively assess the effect of conditions that modulate neuronal MT organization.

References:
[1] Zhang (2017) Molecular biology of the cell, 28, 333-345.
[2] Elek (2020) IEEE Transactions on Visualization and Computer Graphics, 27, 806-816.

Poster 03

Impact of small molecules and peptides on Tau-Microtubules interaction and Tau aggregation in primary peripheral neurons, and models of Alzheimer's disease

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Tau protein, a member of the microtubule associated proteins, is found in abundance within the vertebrates' neuronal axons. In fact, Tau is involved in the regulation of microtubules (MTs) polymerization, it is estimated that almost 80% of Tau has close interaction with neuronal MTs.
Different Tau conformational changes can lead to pathological Tau aggregations causing tauopathies, which may result in neurodegenerative diseases development, e.g., Alzheimer's disease. Hence, we conducted a comprehensive study relating Tau, MTs assembly and neuronal dysfunction using known and novel small molecules. These molecules were examined through in vitro experiments on two model neuronal cells: PC12 cells and mice dorsal root ganglia neurons primary culture. Tau protein (Tau-441wt) was transfected/transduced into the neurons with a green fluorescent protein tag. Tau-MTs interaction was monitored in real time using live cell imaging. Moreover, we assessed the small compounds' neuroprotection and cytotoxicity potentials on a human neuroblastoma cell line, SH-SY5Y. In addition, we determined the tendency of those compounds to cross the blood brain barrier in an in vitro model. Last, we quantified the alterations in MTs structure upon the presence of Tau and/or small molecules using light beam X-ray fiber diffraction. This study allowed us to determine the dynamic changes in Tau-MTs interaction due to the presence of Tau aggregates and/or the presence of different small molecules, as Zampanolide.
This study meets the combined quest of unravelling a small compound's effect on the structural level of MTs with providing the biological proof through cell line and primary culture neuronal models. Consequently, we could determine small molecules' activity relationship with Tau protein and the implicated MTs' dynamics alterations.

Poster 04

Tau shapes microtubule dependent transport in the dendrites

Marina Rierola[1], Nanci Monteiro-Abreu[1], Nataliya I. Trushina[1], Michael Holtmannspötter[2], Rainer Kurre[2], Jürgen J. Heinisch[3], Roland Brandt[1,4], Lidia Bakota[1]

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Alterations in the microtubule-associated protein tau are thought to play an important role in several neurodegenerative diseases. Therefore, the function of tau in neurons has been extensively studied, particularly in the axon where it is enriched. However, tau is also present in the neuronal dendritic arbor under physiological conditions and might also have a specific function in this compartment. Therefore, in the present study, we aimed to investigate the role of tau in the dendrites of pyramidal neurons of the hippocampus.
Using fluorescence decay after photoactivation approach, we followed the dynamics of microtubules in primary hippocampal neurons of tau KO and control mice. Our results show that the lack of tau causes increased microtubule dynamics. In an authentic neuronal tissue environment, which is ensured by organotypic hippocampal slices, we observed longer dendritic processes with more branches of hippocampal pyramidal cells. Using Lattice Light-Sheet Microscopy, we observed a more processive microtubule-based transport in the dendritic processes of neurons lacking tau. Mass spectrometric analysis revealed no compensatory expression of other microtubule-associated proteins, but greatly increased expression of α-synuclein.
Our data show that tau shapes microtubule-dependent transport in dendrites and influences dendritic arborization, indicating a clear role of tau in this compartment.

Poster 05

Dissecting the effect of extracellular tau on tau in cells:
A new cellular model for understanding tauopathies

Simone Attanasio[1,2], Nicolo Bisi[1,3], Roland Brandt[1]

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Amyloidosis is characterized by protein misfolding and the formation of aggregates, such as the neurofibrillary tangles (NFTs) composed of tau protein found in Alzheimer's Disease. In the physiological state, tau is necessary for the correct function and dynamicity of microtubules (MTs), a major component of the cytoskeleton.
Using cell-free aggregation assays, we confirmed that a pathological tau deletion at residue 280 (Tau ΔK280) strongly increases tau aggregation. Through FDAP (Fluorescence Decay After Photoactivation) experiments in model neurons, we showed that TauΔK280 exhibits reduced MT binding in axon-like processes, indicating increased tau aggregation also in the cellular environment.
Extracellular addition of monomeric or aggregated tau decreased the interaction between TauΔK280 and MTs, suggesting enhanced aggregation of cellular tau.
The data show that our cell model helps to dissect the complex molecular pathogenesis of tauopathies and amyloidosis, particularly with regard to identifying the relevant tau species involved in the initiation and propagation of tau aggregation.

References
Arendt, T. et al. (2016), Brain Res. Bull. 126, 238-292.
Brandt, R. and Bakota, L. (2017) J. Neurochem. 143, 409-417.

Poster 06

The axon initial segment is altered in a tau dependent manner in various brain regions of mice

Kerli Tulva[1], Nânci Monteiro Abreu[1], Roland Brandt[1,2,3], Lidia Bakota[1]

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Axonal pathology has been widely studied in Alzheimer's disease (AD). The axon initial segment (AIS) is located at the proximal part of the axon. It generates and organizes action potentials as well as maintains the molecular identity of the axon. Tau plays a major role in neurodegenerative diseases, like Alzheimer's disease. Therefore, our objective was to investigate potential influence of tau on the structure, position and physiological role of AIS. AnkyrinG was used as a marker of the AIS, and laser scanning micrographs were analyzed for the length of the AIS and the distance from the cell body. AIS structure and position was analyzed in primary hippocampal cultures and in young (3M old) and aged (1Y old) TauKO mice, compared to the control strain. AIS was longer in the neurons of the hippocampal primary culture from TauKO mice compared to controls. After introducing full length tau or tau lacking the N-terminal domain to the primary neuronal cells, we found that the AIS length in TauKO mice was restored to the level of the controls. However, tau was not affecting the position of AIS. Interestingly, we observed a shorter AIS in the adult TauKO mice compared to control, which reached significance in all of the four brain regions at 3M of age. Electrophysiological recordings using multi-electrode array (MEA) in the hippocampal CA1 region were taken to characterize the differences in long term potentiation (LTP). These results showed that 3M and 1Y old TauKO mice had higher LTP in the stratum pyramidale. Our data shows that AIS is altered in TauKO mice and exogenously introduced tau restores the AIS structure, suggesting a critical role of tau in the organization of AIS.

Photo Section

Introduction by Prof. R. Brandt (Osnabrück University)

Welcome address by Prof. K.U. Kühnberger,
Vice President for Research and Promotion of Young Talent at Osnabrück University

Lectures and Progress Talks

Lecture 1: Prof. N. Vitale (Université de Strasbourg, France)

Lecture 2: Prof. T.G. Oliveira (University of Minho, Braga, Portugal)

Lecture 3: Prof. H.A. Lashuel (Swiss Federal Institute of Technology, Lausanne, Switzerland)

Lecture 4: Prof. A. Straube (University of Warwick, Coventry, UK)

Lectures and Progress Talks

Lecture 5: Prof. S. Kins (Technical University of Kaiserslautern, Germany)

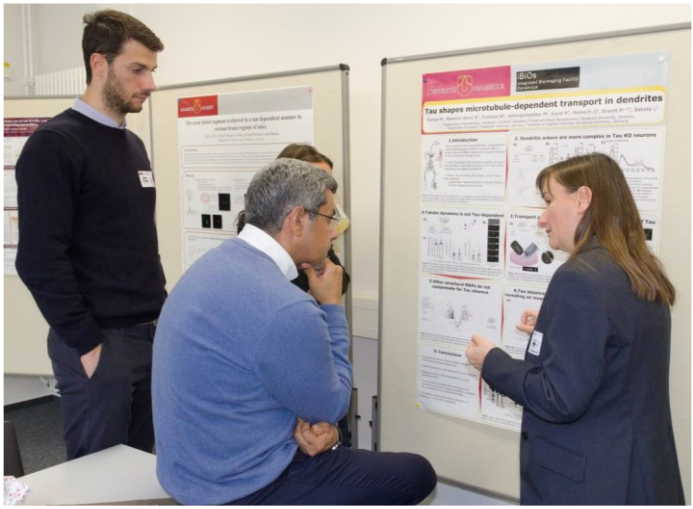
Progress Talk 1: Nataliya Trushina (Neurobiology, UOS)

Progress Talk 2: Kerli Tulva (Neurobiology, UOS)

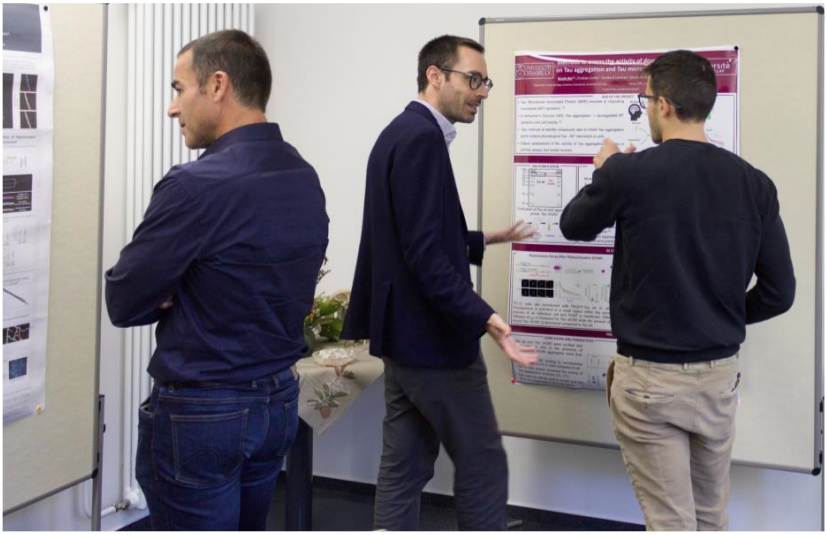
Progress Talk 3: Simone Attanasio (Neurobiology, UOS)

Progress Talk 4: Ahmed Soliman (Neurobiology, UOS)

Poster Presentation and Discussion



Poster Presentation and Discussion



Conversation during Coffee and Buffet

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