UNIVERSITÄT OSNABRÜCK

11th Westerberger Herbsttagung on the Perspectives of Molecular Neurobiology

NEW APPROACHES TO STUDY NEURONS

September 27, 2024
CellNanOS, Osnabrück University

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London, UK

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Progress Talks, Osnabrück

Mahshid Badri Koohi
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Program and further information: www.neurobiologie.uni-osnabrueck.de
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Introduction

We are very pleased to welcome you to the 11th Westerberger Herbsttagung on the Perspectives of Molecular Biology - "New Approaches to Study Neurons". The conference usually takes place every two years and was launched in 2002 on the occasion of the founding of the Department of Neurobiology at Osnabrück University.

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 past Westerberger Herbsttagungen were "Cell and Brain Imaging", "Neural Communication and Dynamics" or "Psychological and Biological Aspects of Explicit and Implicit Memory". Booklets of past Westerberger Herbsttagungen are available on the Department's website (https://www.neurobiologie.uni-osnabrueck.de). Since exciting new experimental approaches to understanding the structure and function of neurons have been developed in recent years, we have decided to focus on "New approaches to studying neurons" and are happy that we have once again managed to bring together a group of excellent researchers from Europe to discuss this year's main theme.

As every time, the Westerberger Herbsttagung is also an opportunity for the Department's early career researchers to present their science to experts in the field. Therefore, I am delighted that we will also have three progress talks and eight posters on selected projects currently being conducted in the Department or presented by former lab members who have recently joined other groups.

The Westerberger Herbsttagung is organized by the entire Department of Neurobiology and I would like to thank everyone for their contribution to the various aspects of organizing such an event. This includes preparing the announcement, planning the program and choosing the buffet. We hope that we can once again create a pleasant atmosphere for fruitful discussions.

A local meeting is also an opportunity for further training for teachers, master students and also offers students at local schools the opportunity to experience live what a scientific meeting is all about. Therefore, I am happy that several teachers, students of cognitive science and also some high school students have found their way to our conference. Welcome and I hope you will enjoy the meeting too.

Finally, I would also like to thank the sponsors of this meeting, especially the local SFB 1557, Nikon Deutschland, which traditionally supports our speaker dinner, as well as Roth, Diagonal and Thermo Fisher Scientific, who regularly support the Westerberger Herbsttagung. We would also like to thank the "Universitätsgesellschaft Osnabrück" for supporting the buffet and the Center for Cellular Nanoanalytics Osnabrück (CellNanOs) and the University for providing rooms and infrastructure.

We are look forward to an exciting conference.

Roland Brandt
(for the organizing committee)

11th Westerberger Herbsttagung on the Perspectives of Molecular Neurobiology

NEW APPROACHES TO STUDY NEURONS

Date: Friday, 27th September, 2024

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

Organizational Committee:

Lisha Singh, Laryssa Alves Borba, Mahshid (Anna) Badri koohi, Kerli Tulva, Lidia Bakota, Roland Brandt, Bettina Flenker, Wilfried Hamann, Vanessa Herkenhoff, Beat Grimmelsmann, Iana Ushko, Henning Borgstädde

Supported by:

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Program

9:00 Introduction and Welcome address: Roland Brandt (Head of the Department of Neurobiology and Dean of the Faculty of Biology/Chemistry, Osnabrück University, Germany)

Session I: (Chair: L. Bakota)

9:15 Lecture 1: Conceição Bettencourt, PhD (University College London, UK):

"Brain DNA methylomics: insights into dysregulated genes and affected cell types in neurodegenerative diseases"

10:00 Lecture 2: Melissa Birol, PhD (Max Delbrück Center, Berlin, Germany)

"Early deviations in cellular lipid profiles and cell-to-cell communication in neurodegenerative disease trajectories"

10:45 Coffee Break

11:15 Lecture 3: Amelie Freal, PhD (Amsterdam UMC, Netherlands):

"Local control of membrane trafficking drives axon initial segment formation and plasticity"

12:00 Progress Talk 1: Lisha Singh and Laryssa Alves Borba (Neurobiologie, UOS):

"Organization of microtubules in the peripheral nervous system: Does tau play a role?"

12:20 Progress Talk 2: Kerli Tulva and Lisha Singh (Neurobiologie, UOS):

"The absence of tau is altering the length but not the distance of the axon initial segment which is rescued by human full-length tau in a microtubule independent manner"

12:40 Progress Talk 3: Mahshid (Anna) Badrikoohi (Neurobiologie, UOS):

“G3BP1: a tunable stress granule organizer?"

12:55 Lunch, Coffee and Poster session

Session II: (Chair: R. Brandt)

15:15 Lecture 4: Clévio Nóbrega, PhD (Algarve Biomedical Center Research Institute, Faro, Portugal):

"Linking stress granules, RNA-binding proteins and neurodegeneration: insights from polyglutamine spinocerebellar ataxias"

16:00 Lecture 5: Elena Taverna, PhD (Human Technopole, Milan, Italy)

"Of human and apes: the cell biology of neurons through the lens of evolution"

16:45 Concluding remarks: R. Brandt

17:00 End of Meeting

Lectures

Lecture 1

Brain DNA methylomics: insights into dysregulated genes and affected cell types in neurodegenerative diseases

Conceição Bettencourt, PhD

University College London, UK

Neurodegenerative diseases encompass a heterogeneous group of conditions characterised by the pathological aggregation of proteins in the brain and progressive degeneration of the structure and function of the central or peripheral nervous systems. Various pathogenic mechanisms are thought to contribute to disease, including aberrant DNA methylation. DNA methylation is the most widely studied epigenetic modification and consists of the addition of a methyl group to the DNA without changing the underlying DNA sequence. Along with other epigenetic modifications, DNA methylation mediates the interplay between the genetic makeup of an individual and their environmental exposures and allows the intricate spatiotemporal control of gene expression, which is crucial for many processes relevant to the brain, including in brain development, learning, memory, and brain cell-type specification. Technological advances that allowed querying DNA methylation throughout the genome, have empowered investigations of relevant candidate genes and epigenome-wide association studies (EWAS) to identify disease-associated DNA methylation alterations at the single nucleotide resolution. Similarly, advances in other high-throughput technologies, such as RNA sequencing, have facilitated the study of other omics, including the study of downstream transcriptomic consequences of DNA methylation changes at a wider scale. Whilst most DNA methylation studies in neurodegenerative diseases employ ‘bulk’ tissue analysis (e.g. bulk brain tissue), some studies exploring cell type-specific DNA methylation changes start to emerge. We will discuss examples of brain and cell type-specific DNA methylation changes in neurodegenerative diseases, including Alzheimer’s disease and Frontotemporal Dementias, and how this can bring new insights into disease biology.

Lecture 2

Early deviations in cellular lipid profiles and cell-to-cell communication in neurodegenerative disease trajectories

Melissa Birol, PhD

Max Delbrück Center, Berlin, Germany

Aberrant lipid metabolism is emerging as a central component, interfacing with all major facets of neurodegenerative diseases (NDs). These include pathological hallmarks of intracellular deposits of intrinsically disordered proteins (IDPs) and their high spatiotemporal pattern of progression through the brain. Currently, it is unclear when and how protein propagation initiates and what defines cellular vulnerability to spread IDPs throughout the brain. This is largely because the physiological functions of several disease-associated IDPs remain unknown, and, this is further challenged by metabolic pathways which may dictate major changes to their function. In our group we question how the redistributed lipidome in Alzheimer's and Parkinson's disease brains affect IDP functions and influence their spreading kinetics both in neurons and in surrounding glia. Our aim is to monitor changes in lipid metabolism and IDP functions which will enable the discovery of common nodes and targetable pathways applicable for early intervention. We probe quantitative cell biology by developing and integrating state-of-the-art microscopy technologies to detect and monitor the evolution of early-stage disease phenotypes. We combine systems level -omics approaches with functional calcium and metabolic imaging to elucidate how misregulation of cellular crosswalks within the brain insult neuronal function. We address these questions on different biological scales - from molecular studies to patient derived induced pluripotent stem cell 2D co-/cultures and 3D brain organoid systems, to map early events that initiate propagation trajectories and connect modulation of specific lipid metabolic pathways to IDP-induced proteinopathies..

Lecture 3

Local control of membrane trafficking drives axon initial segment formation and plasticity

Amelie Freal, PhD

Amsterdam UMC, Netherlands

The axon initial segment (AIS) is a neuronal compartment located at the base of the axon and is critical for action potentials generation and maintenance of neuronal polarity. Activity-dependent plasticity of the AIS allows neurons to adapt action potential output to changes in network activity. Action potential initiation at the AIS highly depends on the local density and distribution of voltage-gated sodium channels, but the molecular mechanisms regulating their AIS localization and plasticity remain largely unknown.

We showed that membrane protein accumulation during AIS formation relies on the cooperation between axonal transport and local control of endocytosis. Moreover, we developed genetic tools to label endogenous sodium channels and their scaffolding protein Ankyrin-G, to reveal their nanoscale organization and longitudinally image AIS plasticity in hippocampal neurons both in slices and primary cultures. Interestingly, we find that N-methyl-D-aspartate receptor activation causes both long-term synaptic depression and rapid internalization of AIS sodium channels within minutes. The internalization of sodium channels occurs preferentially in the distal part of the AIS and depends on clathrin-mediated endocytosis. Moreover, this activity-driven AIS shortening AIS increases the threshold for action potential generation. These data reveal a fundamental mechanism for rapid activity-dependent AIS reorganization and suggests that plasticity of intrinsic excitability shares conserved features with synaptic plasticity.

Lecture 4

Linking stress granules, RNA-binding proteins and neurodegeneration: insights from polyglutamine spinocerebellar ataxias

Clévio Nóbrega, PhD

Algarve Biomedical Center Research Institute, Faro, Portugal

Spinocerebellar ataxias (SCA) are a group of heterogenous neurodegenerative diseases (NDs) characterized mainly by a neurodegeneration in the cerebellum. A specific subset of this group is the polyglutamine SCA, which includes 6 different diseases. These conditions arise from the expansion of the CAG trinucleotide repeat within the coding regions of the causative genes. This abnormal expansion triggers conformational alterations in the protein structure, leading to the formation of pathological aggregates, which are a main feature of these diseases.

Currently, it is widely recognised and accepted that the molecular events underlying molecular pathogenesis are complex. Recently, stress granules (SGs) and their components emerged as important players in the context of NDs. SGs are membraneless cell compartments formed in response to different stress stimuli, wherein translation factors, mRNAs, RNA-binding proteins (RBPs) and other proteins coalesce together.

In this presentation I will present some results on the possible implication of SGs, RBPs in the polyglutamine SCA, as players involved in the neurodegenerative process, but also as possible targets for therapeutic development.

Lecture 5

Of human and apes: the cell biology of neurons through the lens of evolution

Elena Taverna, PhD

Human Technopole, Milan, Italy

Differences in cognitive abilities between humans and non-human primates are thought to depend on greater numbers of neurons and more complex neural architecture and functions in humans. To study the cellular and cell biological basis of evolutionary differences, we generated induced excitatory neurons (iNeurons, iNs) from chimpanzee, bonobo, and human stem cells by expressing the transcription factor neurogenin-2 (NGN2). Single-cell RNA sequencing showed that genes involved in dendrite and synapse development are expressed earlier during iNs maturation in the chimpanzee and bonobo than the human iNs. The transcriptional differences result in striking differences in the timing and dynamics of functional maturation. Indeed, chimpanzee and bonobo iNs showed more repetitive action potentials and more spontaneous excitatory activity than human iNs at any time point considered.

Our data point to the timing of synapse maturation as a possible driver of functional differences between human and apes' neurons and raise the intriguing possibility that dynamics of maturation might influence overall brain function. We are currently studying the cell biology of synapse evolution with a multimodal, holistic approach spanning from genomics analysis to cell biology, electron microscopy and calcium imaging. Our ultimate goal is to correlate the diversity of cell biological processes in apes neurons with their with functional output and network properties.

Progress Talks

Progress Talk 1

Organization of microtubules in the peripheral nervous system: Does Tau play a role?

Lisha Singh and Laryssa Alves Borba

Department of Neurobiology, Osnabrück University, Germany

In neuronal axons, microtubules exhibit a unique organization consisting of an array of relatively short fragments, yet the mechanisms regulating this structure remains unclear. Tau, a microtubule associated protein, known to interact with microtubules and involved in signalling and regulation processes and its dysfunction is linked to neurodegenerative disorders known as tauopathies.

To explore tau's role in microtubule organization, we employed DNA-PAINT (Point Accumulation in Nanoscale Topography), a high resolution, single-molecule localization microscopy technique. Initially, 2-D algorithmic image analysis allowed us to visualize microtubule lattice organization in cultured DRG (Dorsal root ganglion) neurons, revealing a significant reduction ~20 % reduction in microtubule length and increased microtubule density in tau knock-out mice, without changes in total microtubule mass. These findings give useful information to unfold the role of tau in spatial arrangement of microtubules within axons.

However, to further validate and expand upon these observations, we implemented a 3D analysis approach. While conventional software such as SIFNE (SMLM Image Filament Network Extractor) limits analysis to 2D, we adapted a new method using Polyphorm, a multi-agent algorithm, along with a custom Python pre- and posy processing script, to extract 3D structural information TIRF microscopic data. This enhanced 3D analysis provides more detailed spatial mapping of microtubule organization promises to be a robust approach.

Progress Talk 2

The absence of tau is altering the length but not the distance of the axon initial segment which is rescued by human full-length tau in a microtubule independent manner

Kerli Tulva & Lisha Singh

Department of Neurobiology, Osnabrück University, Osnabrück, Germany

The axon initial segment (AIS) is located at the proximal part of the axon where the action potentials are generated. It contributes to the molecular identity of the axon also enabling enrichment of tau within the process. Tau plays a major role in neurodegenerative diseases, like Alzheimer’s disease where axonal pathology has been widely studied. Therefore, our objective was to investigate potential influence of tau on the structure and position 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 were analyzed in primary hippocampal cultures of tauKO and B6 (control) mice. Our data shows that AIS was longer in the neurons of tauKO mice compared to control and increased in both genotypes as the culture aged, whereas the position got closer to the soma in later developmental stage however, this change was not accompanied by differences in the genotype. 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. Interestingly, the fetal form of tau did not rescue the length of the AIS. The position of AIS was not affected by any of the tau isoforms. Super-resolution imaging of the microtubules showed surprisingly no difference between the genotypes. In addition, the distribution of voltage-gated sodium channels (PanNav as a marker) was investigated, where the length distribution within the AIS was much less affected. 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. However, microtubules are not affected, pointing towards a different mechanism, and not tau microtubule interaction for of the alterations of AIS in the absence of tau.

Progress Talk 3

G3BP1: a tunable stress granule organizer?

Mahshid (Anna) Badri Koohi

Department of Neurobiology, Osnabrück University, Osnabrück, Germany.

G3BP1 (Ras-GTPase-activating protein SH3 domain-binding protein 1) is a critical component of stress granules, cytoplasmic aggregates of proteins and RNA that form in response to cellular stress. G3BP1 functions as a molecular switch, regulating the assembly and disassembly of stress granules through its RNA-binding activity and stress-sensing properties. This study investigates the role of specific phosphorylation sites on G3BP1 in the dynamics of neuronal stress granules under different stress factors.

Using a phosphoproteomic approach, we identified four sites that are phosphorylated in G3BP1 of model neurons with different stressors. We utilized PAGFP (photoactivatable green fluorescent protein)-tagged G3BP1 constructs, including wild-type (PAGFP-G3BP1wt) and non-phosphorylatable mutants of the four sites (PAGFP-G3BP1 S149A, S230-S232A, S253A), to analyze the effects of these mutations on stress granule dynamics in living neuronal cells. Fluorescence Decay After Photoconversion (FDAP) experiments were conducted to measure the distribution and mobility of G3BP1 in response to different stressors, including sodium arsenite and hydrogen peroxide.

We observed that about 50% of exogenously expressed PAGFP-G3BP1wt was present in arsenite-induced stress granules with a time constant of ~230 s ($t_{1/2}$~160 s). We did not observe any significant changes in the non-phosphorylatable G3BP1 mutants or with H_2O_2 as an alternative stressor. Ourfindings therefore demonstrate that the phosphorylation state of G3BP1 does not significantly affect its ability to localize to stress granules or to alter the dynamic shuttling between them. The results suggest that although G3BP1 is phosphorylated at multiple sites under stress conditions, differential phosphorylation of G3BP1 does not have a major regulatory function in determining the formation or dynamics of neuronal stress granules.

Poster Abstracts

Poster 1

Predicting protein solubility upon single mutation leveraging sequence and structure information

Simone Attanasio, Fabrizio Pucci, Marianne Rooman

Computational Biology and Bioinformatics, Université Libre de Bruxelles, Brussels, Belgium

Protein solubility is a crucial property in molecular biology, biotechnology, and medicine due to its implications for protein function, purification, and administration, particularly in antibody-based therapeutics. This work presents a novel approach called SOuLMuSiC to predict the effect of mutations on protein solubility, integrating sequence and structural information. A dataset of mutations with known solubility values was manually curated from the original literature. Each mutation's effect on solubility was categorized into five different classes from highly insoluble to highly soluble. The constructed dataset, containing about 700 mutations, enabled the training of SOuLMuSIC, our artificial neural network model that combines different features such as the folding free energy of the wild-type protein computed with a series of statistical potentials and some basic biophysical characteristics of residues such as the hydrophobicity. SOuLMuSiC performs well in strict cross-validation on the aforementioned training set as well as when validated using additional data on mutation solubility from high-throughput experiments. Unlike other solubility predictors solely relying on sequence information like CAMSOL and SODA, this model, integrating protein 3D structures, enhances our understanding of how structural information affects protein solubility. SOuLMuSiC opens avenues for precision protein engineering applications aimed at improving the solubility of the target proteins and will be available for academic use at dezyme.org.

Poster 2

G3BP1: a tunable stress granule organizer?

Mahshid (Anna) Badri Koohi, Nanci Monteiro-Abreu, Nataliya I. Trushina, Roland Brandt

Department of Neurobiology, Osnabrück University, Osnabrück, Germany.

This study investigates the role of G3BP1 phosphorylation in neuronal stress granule (SG) formation and dynamics using live cell imaging of model neurons. Stress granules are membrane-less organelles formed in response to cellular stress, and G3BP1 is crucial for their nucleation, assembly, and regulation. We employed mass spectrometry-based phosphoproteomics to identify stress-induced phosphorylation changes in G3BP1 and different stress conditions (heat shock, sodium arsenite, and hydrogen peroxide).

We identified four sites in G3BP1 that were phosphorylated under different stress conditions. Two of the sites (S229 and S231) were significantly less phosphorylated in the presence of hydrogen peroxide compared to control conditions. To analyze the dynamics of G3BP1 within stress granules, we used Fluorescence Decay After Photoconversion (FDAP) with PAGFP-tagged human G3BP1 and non-phosphorylatabel mutants of the corresponding phosphorylation sites. Using this approach, we were able to quantify the extent to which exogenously expressed G3BP1 localizes to stress granules and determine its shuttling frequency between stress granules. Our findings indicate that the phosphorylation state of G3BP1 does not significantly impact its localization to stress granules nor its shuttling between stress granules. This suggests that although G3BP1 is phosphorylated under stress conditions, its phosphorylation state at these sites is not a crucial factor in regulating its dynamic behavior.

Poster 3

Discovery of PHOX15, a polypharmacological drug candidate able to restore physiological tau-microtubules interaction for the treatment of Alzheimer's disease

N. Bisi[a,b], L. Pinzi[a], C. Conze[b], G.D. Torre[a], A. Soliman[b], N. Monteiro-Abreu[b], N.I. Trushina[b], A. Krusenbaum[b], M.K. Dolouei[b], A. Hellwig[c], M.S. Christodoulou[d], D. Passarella[d], L. Bakota[b], R. Brandt[b], G. Rastelli[a]

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[b]Department of Neurobiology, Osnabrück University, Osnabrück, Germany,
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Tauopathies are defined as neurodegenerative diseases characterized by the aggregation of the tau protein.[1] Physiologically, tau is involved in the regulation of microtubules (MTs) dynamics and function.[2] However, under pathological conditions (e.g., hyperphosphorylation, mutation), tau can dissociate from MTs to assemble into aggregates responsible for neurodegeneration.[1,3] Therefore, the design of compounds preventing tau aggregation and restoring the physiological tau-MTs interaction represents a promising strategy for tauopathies treatment. In the search of compounds endowed with such properties, we first performed extensive *in silico* analyses, identifying a series of 2-phenyloxazole (PHOX) derivates as potential candidates for the treatment of Alzheimer's disease, the most prevalent and spread tauopathy. To assess the candidates' activity, we then performed extensive experimental analyses, and developed a novel *in vitro* cellular assay that enables to monitor pathological changes in human tau. These analyses allowed us to identify one candidate (PHOX15) able to restore tau-MTs interaction and to reduce tau aggregation in living neurons.[4] Finally, *in silico* structure-based simulations allowed us to identify a series of cryptic pockets in tau cryo-EM structures, one of which being potentially involved in the PHOX15 binding.[4]

Acknowledgment: The research leading to these results has received funding from the European Union—NextGenerationEU through the Italian Ministry of University and Research under PNRR—M4C2-I1.3 Project PE_00000019 "HEAL ITALIA"

References: [1] K.A. Josephs, Mayo Clin. Proc., 2017, 92, 1291-1303. ; [2] J. Avila, J.J. Lucas, M. Perez, F. Hernandez, Physiol. Rev., 2004, 84, 361-384. ; [3] M.E. Orr, A.C. Sullivan, B. Frost, Trends Pharmacol. Sci., 2017, 38, 637-648.; [4] L. Pinzi, C. Conze, N. Bisi, G.D. Torre, A. Soliman, N. Monteiro-Abreu, N.I. Trushina, A. Krusenbaum, M.K. Dolouei, A. Hellwig, M.S. Christodoulou, D. Passarella, L. Bakota, G. Rastelli, R. Brandt, Nat. Commun. 2024, 15, 1679.

Poster 4

3D DNA-PAINT: A new approach to analyze microtubule arrays

Laryssa Alves Borba[1], Lisha Singh[1], Niklas Alvar Laasch, Roland Brandt[1, 2, 3]

[1]Department of Neurobiology, [2]Center for Cellular Nanoanalytics, [3]Institute of Cognitive Science, Osnabrück University, Germany.

The local organization of axonal microtubule arrays may play an important role in regulating axonal transport. However, axonal microtubules are densely packed, making their resolution impossible using classical light microscopic techniques. DNA points accumulation for imaging in nanoscale topography (DNA-PAINT) provides a localization-based method for super-resolution microscopy. When paired with TIRF microscopy, it generates high-resolution 3D data, by utilizing short dye-labeled oligonucleotides that bind to a target docking strand, causing the dye to blink upon laser excitation. With the aggregation of these blinking events, the molecule's position can be determined with nanometer resolution from the point spread function (PSF) produced by the microscope. The PSF arises from the astigmatic lens system, which produces two distinct focal planes. When a fluorescent particle resides within the focal plane, its PSF manifests as a circular pattern. Conversely, if the particle is situated vertically above the focal plane, the PSF becomes elliptical in the horizontal dimension; if located below the focal plane, it appears elliptical in the vertical dimension. This generates 3D super-resolution images of target molecules, which can be further analyzed using a three-dimensional approach known as 3D DNA-PAINT. To analyze the 3D data, a natural growth algorithm called Monte Carlo Physarum Machine (MCPM) is adopted. The algorithm is implemented in a program called Polyphorm which utilizes a multi-agent framework to connect and establish a stable equilibrium among the provided data points, and in conjunction with a Python Pre- and Postprocessing script, extract filaments from total internal reflection fluorescence (TIRF) microscopy data. Both DNA-PAINT and 3D DNA-PAINT techniques yield multiple outputs, facilitating quantitative comparative analyses. Notably, the new 3D methodology significantly enhances the visualization of junctions. The incorporation of the third dimension provides insights into genuine junctions, as opposed to those that may only be discernible in maximum intensity projections. It also enables the use of greater slice depths in microscopy data and may allow the measurement of distances between filaments. A comparative analysis was done by comparing the results from DRG neurons of one-year-old B6 and tau KO mice, utilizing the same data in both filament extraction programs. Initial evaluations of the Polyphorm program have shown promising results, indicating that further refinements of this method could enhance the comprehensive analysis of microtubule arrays in model neuronal systems.

Poster 5

Microtubule Organization in Dorsal Root Ganglion (DRG) Neurons: Unravelling the Pathways of Ageing and Disease

Lisha Singh[1], Michael Holtmannspötter[2], Nataliya I. Trushina[1], Roland Brandt[1,2]

[1]Department of Neurobiology, and [2]Center for Cellular Nanoanalytics, Osnabrück University, Germany

In neuronal axons, microtubules exhibit a unique organization consisting of an array of relatively short fragments. However, it is unclear how this organization is regulated and maintained.

We used DNA-PAINT (Point Accumulation in Nanoscale Topography), a single-molecule localization microscopy technique, followed by algorithmic image analysis to determine the three-dimensional arrangement of the microtubule lattice in axons of cultured DRG neurons. In particular, we analyzed the effect of tau protein, a neuronal microtubule-associated protein that is enriched in the axons of mature neurons and contributes to a class of neurodegenerative diseases collectively referred to as tauopathies.

We report that in DRG neurons from tau knockout mice, mean microtubule length is reduced by ~20% and microtubule density is increased without any discernible change in microtubule mass. Region-specific analysis revealed that microtubule crowding, defined by a high proportion of microtubules in close proximity, was increased particularly in the middle region of the axon of DRG neurons prepared from tau knockout animals.

Our data demonstrate that DNA-PAINT is a powerful tool to quantitatively assess changes in the axonal microtubule array. They point out that tau acts as a regulator of microtubule organization, specifically by ensuring proper spacing of microtubules in the axon shaft.

Poster 6

Loss of Tuba4a C-terminal polyglutamylation interferes with microtubule-tau interaction: An in-cell approach

Lisha Singh[1], Torben J. Hausrat[4], Lidia Bakota[1], Roland Brandt[1,2,3]

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The microtubule cytoskeleton is essential for a variety of cellular processes in eukaryotes, forming dynamic structure composed of alpha- and beta-tubulin heterodimers. Tubulin heterogeneity arises from post-translational modifications (PTMs), which combined to form the 'tubulin code' signal interpreted by microtubule-associated proteins (MAP) and motor proteins. Tau, a key MAP, is enriched in axons of mature neurons, when aggregated in pathological condition becomes a hallmark of disease progression in tauopathies. Tau interaction with microtubules via kiss-and-hop mechanism, if disturbed may initiate the pathological changes.

To investigate the molecular mechanisms underlying this process, we generated a knock-in mice with specific loss of Tuba4a c-terminal polyglutamylation. This modification is critical for regulating tau's binding affinity to microtubules. Using in-cell, florescence after photoactivation (FDAP) approach on hippocampal neuronal culture from wildtype (+/+) and knock-in (p/p) mice, we analyzed the impact of this loss on tau-microtubule interaction dynamics.

Our results reveal that loss of Tuba4a c-terminal polyglutamylation significantly decreases the dynamicity of tau binding to microtubules, as indicated by reduced association (k^*_{on}) and dissociation (k^*_{off}) rate constants. FDAP scatter plots shows a clear decrease in both parameters, suggesting impaired tau-microtubule interaction dynamics in neurons from p/p mice. Furthermore, mathematical modeling of the decay curves supports a reduction in the overall dynamic interaction of tau with microtubules, which may ultimately affect the efficiency of axonal transport.

Poster 7

The axon initial segment is altered in a tau dependent manner in the hippocampus, cortex and amygdala in mice

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

[1]Department of Neurobiology, [2]Center for Cellular Nanoanalytics, [3]Institute of Cognitive Science, Osnabrück University, Osnabrück, Germany

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 AD. Therefore, our objective was to investigate potential influence of tau on the structure and position of the 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. In adult mice AIS was also analyzed in the cortex and amygdala. 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. 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.

Poster 8

Chronic inflammation induced by TNF-α causes loss of dendritic spines in a tau independent manner and is reversed by EpoD

Kerli Tulva[1], Laryssa Alves Borba[1], Nataliya Trushina[1], Lidia Bakota[1], Roland Brandt[1,2,3]

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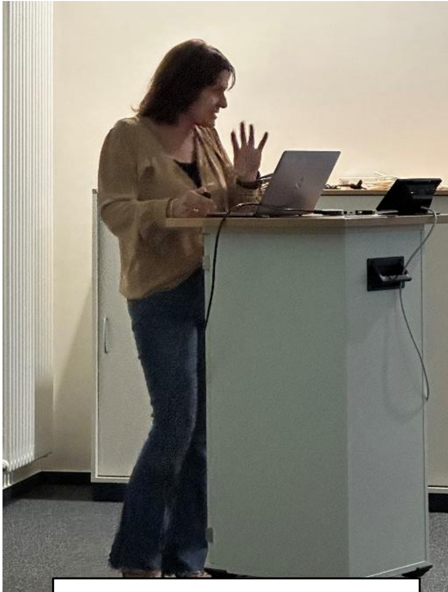
Alzheimer disease (AD) is a progressive neurodegenerative disorder with two histopathological hallmarks, the accumulation of Aβ peptides and intracellular tau fibrils. In recent years it has been found that inflammation has a key impact in the development of the disease. Tumour necrosis factor α (TNF-α) is the major cytokine causing inflammation in AD. In this study the effect of chronic treatment with TNF-α is investigated on synaptic connectivity in hippocampal organotypic slices in presence or absence of tau using 23auKO and B6 (control) mouse models. The hippocampal tissue slices were treated for 24 hours with 50µg/ml TNF-α. Analysis of confocal laser scanning micrographs of dendritic segments were showing a decrease in spine density after 24 hours of TNF-α treatment in both genotypes, indicating that tau has no specific effect on this spine parameter. Even though there was no tau effect, EpoD, which is a microtubule stabilizing agent, reversed spine density, indicating the involvement of microtubules in chronic TNF-α treatment induced dendritic spine modulation. Moreover, mass spectrometry results showed that there was an up-regulation of microtubule related kinesins. The morphology of neurons was not influenced by TNF-α treatment. Our data suggests, that inflammation induced by chronic TNF-α treatment affects dendritic spines via tau independent microtubule modulation.

Speakers Dinner: Hotel Walhalla

Lectures and Progress Talks

Clévio Nóbrega (Faro)



Elena Taverna (Milan)

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Conceição Bettencourt (London)

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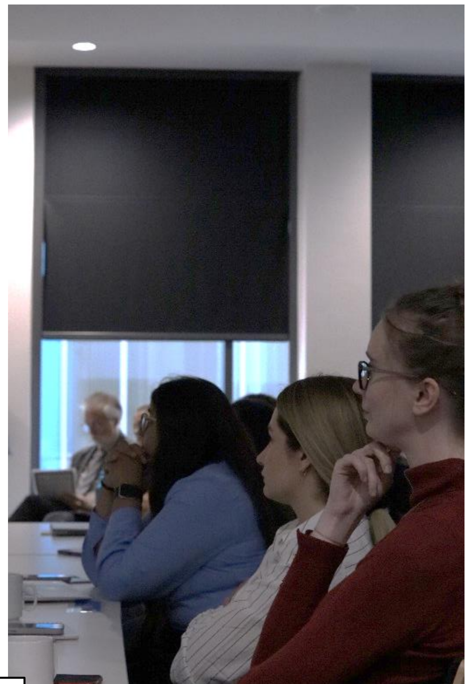
Amelie Freal (Amsterdam)



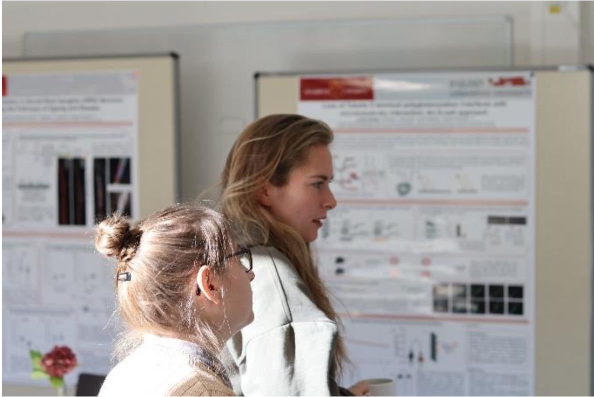
Mahshid (Anna) Badrikoohi (Osnabrück)

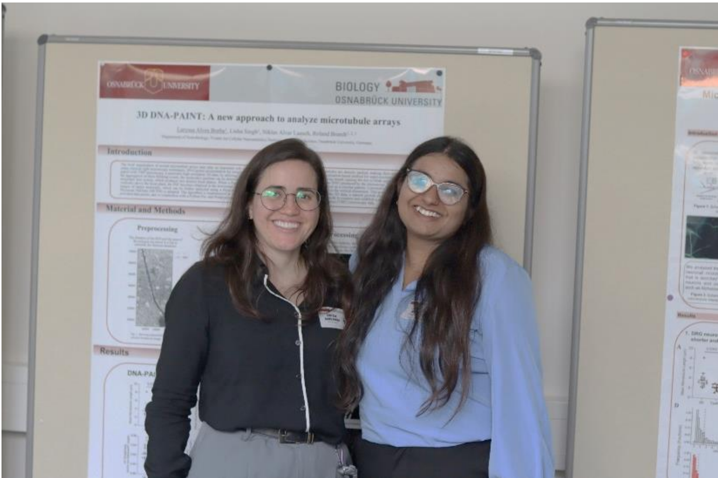
Lectures and Progress Talks

Laryssa Alves Borba and Lisha Singh (Osnabrück)

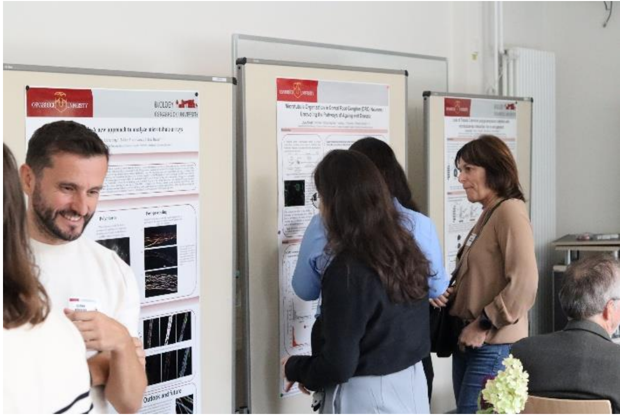
Kerli Tulva and Lisha Singh (Osnabrück)

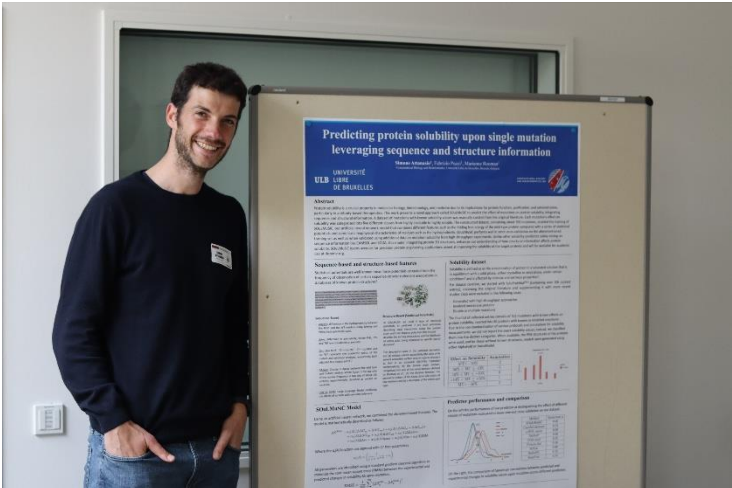
Poster Presentation and Discussions





Poster Presentation and Discussions





Poster Presentation and Discussions

Conversations during Coffee and Buffet

Conversations during Coffee and Buffet

Conversations during Coffee and Buffet

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Conversations during Coffee and Buffet

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