Leibniz Gemeinschaft

Final Report
Leibniz Competition

A Synaptoneurolipidomics View on Neuronal Plasticity in Insulin Resistance and Alzheimer's disease

Application number: K209/2016

Period under review: 01.01.2017- 30.06.2021 (incl. cost-neutral extension)

Leibniz Institute in charge: Leibniz-Institut für Analytische Wissenschaften – ISAS - e.V.

Project leader: Prof. Dr. Albert Sickmann

Executive Summary

In this project we introduced Synaptoneurolipidomics to the field of synapse biology and by investigating the synaptic status under healthy conditions to define the baseline in order to describe diseases states with altered composition that contribute to synaptic dysfunction and altered signaling. The main focus was the development of a cutting-edge biochemical purification technique to isolate neuronal membranes and membrane subcompartments and provide an inventory of their lipid composition. This included to develop and apply a top-notch extraction workflow that will deliver lipids, metabolites and protein abundance from the very same sample. To merge then the quantitative mass spectrometry and biochemistry data into a network model to understand the complexity of the lipid metabolism at synapses with the aim to generate new hypothesis. Finally, the developed workflow was applied to different models to investigate the alteration of the lipid metabolism compared to normal synaptic function and a high fat diet.

Contents

1. Achievement of objectives and milestones

In this project we introduced Synaptoneurolipidomics to the field of synapse biology and by investigating the synaptic status under healthy conditions to define the baseline in order to describe diseases states with altered composition that contribute to synaptic dysfunction and altered signaling. The main milestones were the development of i) a cutting-edge biochemical purification technique to isolate neuronal membranes and membrane subcompartments and provide an inventory of their lipid composition. To develop and apply ii) a top-notch extraction workflow that will deliver lipids, metabolites and protein abundance from the very same sample. The quantitative mass spectrometry and biochemistry data are iii) merged into a network model to understand the complexity of the lipid metabolism at synapses. In aim iv) the developed workflow was applied to different models to investigate the alteration of the lipid metabolism compared to normal synaptic function and a high fat diet.

2. Activities and obstacles

During the project, a comprehensive workflow for Synaptoneurolipidomics was successfully established to unravel alteration of the lipid metabolism at synapses to study synapse function at various different levels of synapse organization. In addition, the intertwining of additional omics levels such as proteomics and metabolomics into the workflow was proven to beneficial and actually highly supportive for data interpretation. The developed techniques are currently further used within other projects applied to investigate the metabolic syndrome in different mouse models. The project worked out as planned with the additional challenge of career dynamics: two postdocs who had received offers from outside (a measure of success in itself) however, could quickly be replaced ensuring continuity of the project. It was also possible to achieve a cost-neutral prolongation of the support by one year also in part due to the COVID-19 pandemia.

3. Results and successes

Synaptic junctions are the central building blocks of a chemical synapse, and compelling evidence points to an essential function of lipids in synaptic neurotransmission. Trace lipids, including phosphatidylinositol phosphates (PIP) and negatively charged phosphatidylserine (PS) are crucial for multiple steps of the synaptic vesicle cycle and vesicle fusion. Lipids also play a central role in other synaptic processes including the formation and shaping of membrane (Chernomordik and Kozlov, 2003[1]; Puchkov and Haucke, 2013[2]), lipid mediated signal transmission (Piomelli et al., 2007[3]), as well as endocytosis (Kononenko and Haucke, 2015[4]). Since most lipid mediators are direct or indirect products of polyunsaturated phospholipids, sufficient molar content of complex polyunsaturated lipids must be located at synaptic junctions to guarantee their formation (Han, 2007[5]). In addition, several lines of evidence suggest that the lipid composition of synapses might be dynamic (Martin et al., 2014[6]). The existence of more than 40 different lipids known to modulate signaling and to

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influence membrane geometry of synapses and synaptic vesicles (Dieterich and Kreutz, 2016[7]) demands a systematic large-scale study of membrane lipids and their metabolism. In our SAW grant, we provided a quantitative lipid inventory of mouse and rat synaptic junctions. To this end, we developed a multiomics extraction and analysis workflow to probe the interplay of proteins and lipids in synaptic signal transduction from the same sample. Based on this workflow, we generate hypotheses about novel mechanisms underlying complex changes in synaptic connectivity elicited by environmental stimuli. As a proof of principle, this approach reveals that in mice exposed to an enriched environment, reduced endocannabinoid synthesis and signaling is linked to increased surface expression of α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid receptor (AMPAR) in a subset of Cannabinoid-receptor 1 positive synapses. This mechanism regulates synaptic strength in an input-specific manner. Thus, we established a compartment-specific multiomics workflow that is suitable to extract information from complex lipid and protein networks involved in synaptic function and plasticity.
In addition, the workflow was applied to a transgenic mouse model of high-risk aging. The metabolic syndrome is a consequence of modern lifestyle that causes synaptic insulin resistance and cognitive deficits and that in interaction with a high amyloid load is an important risk factor for Alzheimer's disease. It has been proposed that neuroinflammation might be an intervening variable but the underlying mechanisms are currently unknown.
As part of the last work package we first utilized primary neurons to induce synaptic insulin resistance as well as a mouse model of high-risk aging that includes a high amyloid load, neuroinflammation, and diet-induced obesity to test hypotheses on underlying mechanisms. We were able to report that neddylation and subsequent activation of cullin-RING ligase complexes induce synaptic insulin resistance by ubiquitylation and degradation of the insulin-receptor substrate IRS1 that organizes synaptic insulin signaling. Accordingly, inhibition of neddylation preserves synaptic insulin signaling and rescues memory deficits in mice with a high amyloid load, which were fed with a 'western diet'. Collectively the data suggest that neddylation and degradation of the insulin-receptor substrate is a nodal point that links high amyloid load, neuroinflammation, and synaptic insulin resistance to cognitive decline and impaired synaptic plasticity in high-risk aging.

Publications

Borgmeyer M, Coman C, Has C, Schött HF, Li T, Westhoff P, Cheung YFH, Hoffmann N, Yuanxiang P, Behnisch T, Gomes GM, Dumenieu M, Schweizer M, Chocholoušková M, Holčapek M, Mikhaylova M, Kreutz MR, Ahrends R. Multiomics of synaptic junctions reveals altered lipid metabolism and signaling following environmental enrichment. Cell Rep. 2021 Oct 5;37(1):109797. doi: 10.1016/j.celrep.2021.109797. PMID: 34610315.
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Presentation: Computational modeling of sphingolipid metabolism in insulin resistance having mouse model. Canan Has, C Coman, P Westhoff, T Li, HF Schött, AD Confettura, MR Kreutz, R Ahrends. EMBL-EBI Wellcome Trust Workshop, June 2018.

Presentation: SIMPLEX: A multiomics tool for screening of modified hipppocampal signaling pathway. HF Schött, R Ahrends, C Coman, P Westhoff, C Has, T Li. 48. Jahrestagung des Arbeitskreises „Klinischer Lipidstoffwechsel“, Nov 2018, Maikammer, Germany.

Poster: SIMPLEX: A multi-omics approach for screening of modified hippocampal lipid signalling pathways triggered by lifestyle conditions. HF Schött, R Ahrends, C Coman, C Has, P Westhoff, T Li. 52nd Annual Meeting of the German Society for Mass Spectrometry (DGMS), March 2019, Rostock, Germany.

Poster: SIMPLEX: A multi-omics approach for screening of modified hippocampal lipid signalling pathways triggered by lifestyle conditions. HF Schött, R Ahrends, C Coman, C Has, P Westhoff, T Li. 60th International Conference on the Bioscience of Lipids, June 2019, Tokyo, Japan.

Poster: Identification of endocannabinoid-like lipids by nano-liquid chromatography high resolution mass spectrometry in mouse organs. HF Schött, R Ahrends, C Coman, T Li, P Westhoff, C Has, MR Kreutz. 9th European Network of Oxysterol Research (ENOR): Metabolism and Oxysterols; Therapeutics for Lifelong Health, Sep 2019, Edinburgh, United Kindgdom.

Poster: Identification of endocannabinoid-like lipids by nano-liquid chromatography high resolution mass spectrometry in mouse organs. HF Schött, R Ahrends, C Coman, T Li, P Westhoff, C Has, MR Kreutz. 5th Lipidomics Forum, Nov 2019

Poster: Proteome and Phosphoproteome Mapping of Proteins Specificity in Different Subcellular Membrane Fractions of Rat Brain. T Li, AD Confettura, C Has, MR Kreutz, A Sickmann, R Ahrends. 53rd Annual Meeting of the German Society for Mass Spectrometry (DGMS), March 2020, Münster, Germany.

Qualifications of involved personnel

Dr. Robert Ahrends	successfully applied to professorship at University of Vienna, starting date 01.01.2020
Dr. Philip Westhoff	successfully applied for a principle investigator position and is heading now the metabolomics core facility at the Heinrich Heine University in Düsseldorf
Dr. Alessandro Dario Confettura	successfully defended his PhD thesis “Molecular underpinnings of high-risk aging: neuronal insulin signaling, amyloidosis and the metabolic syndrome” in 12/2019
Dr. Canan Has	successfully applied to a postdoctoral position as bioinformatician for multiomics data analysis and integration in MPI-CBG Max Planck Institute of Molecular Cell Biology and Genetics and University Hospital Carl Gustav Carus in Dresden

Dr. Hans-Frieder Schött	successfully applied to a postdoctoral position at Singapore Lipidomics Incubator (SLING), Department of Biochemistry, YLL School of Medicine, National University of Singapore
Li Tingting	PhD, not yet finished

Further dissemination

Scientific outreach at conferences -The results of the project have been presented to the scientific community in a form of oral presentations (ELM, 2018, Leipzig; SLING 2018, Singapore; ILS-Annual Meeting, 2021, Regensburg; OEGMBT, 2021, Innsbruck) and poster on many other scientific meetings.

Workshop - 3rd International Symposium Healthy Ageing Health aging, Berlin 2019, topic "Synaptic Ageing - Challenges for Translational Neuroscience"

Press releases – a) Beschäftigte Mäuse sind empathischer Stimulierende Umwelteinflüsse verbessern die kognitive Leistung und das Sozialverhalten von Mäusen, wie neue Untersuchungen zu synaptischen Prozessen zeigen (https://shorturl.at/clpOS). b) How an enriched environment fires up our synapses (https://shorturl.at/puyB0)

Grants - 2nd granted SAW proposal SyMetAge - Post-translational modifications of the synaptic scaffold controlling age-induced memory impairment

Transfer activities: not applicable, the project was focused on fundamental research questions.

Scientific cooperation: the partners LIN and ISAS have intensively collaborated during the project. With the acceptance of the professorship of R. Ahrends at the Faculty of Chemistry at the University of Vienna the cooperation was extended. Both institutions were involved in the Leibniz Research Network Healthy Aging and have been able to network with other participating institutions via this platform.

4. Equal Opportunities

The Leibniz SAW consortium "A Synaptoneurolipidomics View on Neuronal Plasticity in Insulin Resistance and Alzheimer's disease" was established at the Leibniz-Institut für Analytische Wissenschaften - ISAS - e.V. Dortmund and the Leibniz Institute of Neurobiology (LIN), Magdeburg, and therefore implemented the institute's general policy for equal-career management. Equal opportunities were already guaranteed during the recruitment process. Consequently, for described work packages of the consortium, 1 male postdoc and 1 female postdoc; a female and a male PhD student were recruited, educated and coached during the project. The consortium was additionally supported by the Officer for Equal Opportunities at ISAS and the Office for Equal Opportunities and Career Development at the LIN, founded by the FemPower Project of the State of Saxony-Anhalt.

5. Quality assurance

As part of the Leibniz Association, our consortium as well as the institutes themselves are committed to following the rules of good scientific practice. The PhDs and Postdocs supported from the two institutes were specifically coached in the corresponding rule set. This coaching included individual project-related coaching by the respective PI of the subproject, participation at workshops dedicated to general aspects of good scientific practice. Quality control was also a major topic of several task force meetings held at the LIN and ISAS in the context of the institute's management that were attended by the members of

the consortium. The scientific results obtained in the network were published (and further results await publication in the future) in peer-reviewed scientific journals providing (1) independent critical review of scientific content and quality of the work, (2) independent review of the proper implementation of rules of good scientific conduct, and (3) access to results and data for the scientific community.

6. Additional in-kind resources

For ISAS: Lab equipment and technical support by a technician 50% from Technical Service Bioanalytics, infrastructural support in grant management, data management and transfer
For the LIN: One technician 50% of working time supported the project. Running costs of 30.000.- € p.a. were covered by the LIN.

7. Structures and Collaboration

The project was carried out in a tight network of scientific collaborations between the ISAS Dortmund and the LIN Magdeburg. Core competences and technologies in the network were available for all partners.
The ISAS will benefit from this expertise as well as Principal Investigators from the LIN, who were co-applicants on projects headed by the ISAS. An extensive exchange of personnel to broaden the technological competence at both institutions were conducted and researchers were trained in a multidisciplinary setting. The LIN (Magdeburg) and the ISAS (Dortmund) further benefited from this venture due to multiple additive effects which included the access to high-end technology and methods, functional and dietary mice models and integration into national and international networks for neurology and omics technologies with a strong potential for application transfer.

8. Outlook

Currently, we are considering to extend the project to next generation cell-selective metabolic labeling of proteomes to include dynamics of proteostasis, which will add another dimension in complexity. Particularly the analytical study of synapse biology has not been pursued previously at the ISAS but is an important asset for its future research portfolio, since analytical techniques developed here can be applied to develop novel diagnostic tools for other neurological conditions including Parkinson’s disease, epilepsy, myasthenia gravis or muscular atrophies. The future Leibniz Competition will offer both institutions again the unique opportunity to strengthen sustainable collaborative ties, to sharpen their research portfolio and to address in the best tradition of the Leibniz association a scientific problem with societal impact that can only be tackled in a joint effort.