Final Report
Leibniz SAW Project

"The decline of cognitive function in normal aging:
A view from the LIN"

File Reference: **SAW-2014-LIN-2 332**

Funding Period: 01.01.2014 - 31.12.2017

Project Coordinator
Dr. Michael R. Kreutz, Leibniz Institute for Neurobiology (LIN) Magdeburg

Further PIs
Daniela C. Dieterich (LIN/Otto von Guericke University),
Anna Fejtova, Eckart D. Gundelfinger, Martin Heine, (LIN)

In Collaboration with
Leibniz-Institut für Molekulare Pharmakologie (FMP)
Leibniz-Institut für Altersforschung-Fritz Lipmann Institut Jena (FLI)
André Fischer (DZNE Göttingen)
Stephan Sigrist (FU Berlin)

Contents

1. Aims and Milestones

Normal aging impairs cognition, including learning and memory. We believe that this impairment is due to subtle changes in synapses in vulnerable areas of the brain such as the hippocampus, the focal point of a network of cortical areas that are associated with memory function. In this project we want to pursue the hypothesis that alterations in synaptic protein homeostasis significantly contribute to the cognitive decline during aging. To this end we tried to identify key processes contributing to alterations in synaptic protein content during aging, including glia-synapse interactions, protein trafficking and degradation as well as transcriptional control of neuronal excitability. In order to elucidate the relationship between senescent physiology and cognition in aging we have more specifically in collaboration with other Leibniz Institutes

> ➢ revealed how the aging synapse regulates methylation of promoters of genes that are crucially involved in synaptic aging and cognitive decline,
> ➢ studied the regulation of the ubiquitin-proteasome system (UPS) and its role in age-dependent changes in synaptic plasticity,
> ➢ investigated protein metabolism (synthesis and turnover/degradation rates) and protein modifications (neddylation) in aging synapses,
> ➢ assessed to which extent the aging synapse can be rejuvenated when challenged with young neurons or astrocytes,
> ➢ determined the contribution of ion channel gating and number for the decline in intrinsic neuronal excitability and synaptic plasticity.

2. Activities and Obstacles

Aging and cognitive decline are highly correlated in the elderly population even in the absence of neurodegenerative diseases. Despite the high burden for each individual and the society as a whole the molecular, cellular, and behavioral underpinnings of cognitive decline are barely understood. We concentrated during the course of theye studies on synaptic dysbalances involving (i) altered synaptic proteostasis and (ii) altered functionality of the multipartite synapse accompanied by (iii) dysfunctions of the immune system as the core for cognitive decline. We have addressed these themes in a joint effort by a team of molecular/cellular neurobiologists to eventually break ground for innovative intervention strategies. Overall the research work was conducted in very good alignment with the proposed workflow.

3. Results and Achievements

3.1 Neddylation and the genomic response to synaptic activity in aging

In mammals, the information processing in higher cognitive function is intimately linked to spine synapses. Age-associated impairments are likely due to subtle changes in synapses in vulnerable areas of the brain such as the hippocampus, which is the focal point of a network of cortical areas that are associated with memory function. To understand how alterations in synaptic protein homeostasis based on shifts in PTMs can contribute to AMI, transcriptional control of neuronal excitability is one key process. Also, global DNA-hypomethylation is a hallmark of neuronal aging (Bayraktar & Kreutz, 2018) and expression levels of DNA-methyltransferases decline in the aging brain. It is widely believed that rapid and reversible DNA methylation is essential for the stability of long-term memory, but still very little is known about how synaptic signals can induce changes in DNA-methylation to elicit enduring alterations in plasticity-related gene expression (Bayraktar & Kreutz, 2018). DNMT3A1 is the principal de novo methyltransferase in neurons. It is, however, unclear whether synaptic signals control DNMT3A1 activity. We show that nuclear protein levels of DNMT3A1 are tightly controlled by the activation of synaptic NMDA receptors containing the GluN2A subunit. NMDAR signaling enhances neddylation of the E3-ubiquitin ligase Cullin-4B, which in turn ubiquitylates DNMT3A1. Nuclear DNMT3A1 protein levels in CA1 neurons are

reduced following the induction of NMDAR-dependent LTP and following object location learning. Neddylation-dependent degradation of DNMT3A1 results in hypomethylation of the Bdnf IV promoter, increases Bdnf expression, and promotes late-LTP. Inhibition of the NEDD8 pathway interrupts activity-dependent de-methylation of the Bdnf promoter, late-LTP and object location memory. Collectively these data point to a mechanism that allows for the synaptic control of DNMT3A1 protein levels and is well suited to create a time window for reduced de novo DNA-methylation that is concomitant with increased expression of plasticity-relevant genes. Collectively, the data suggest that **targeting neddylation in the aging brain has the potential to rejuvenate activity-dependent gene expression and synaptic function**.
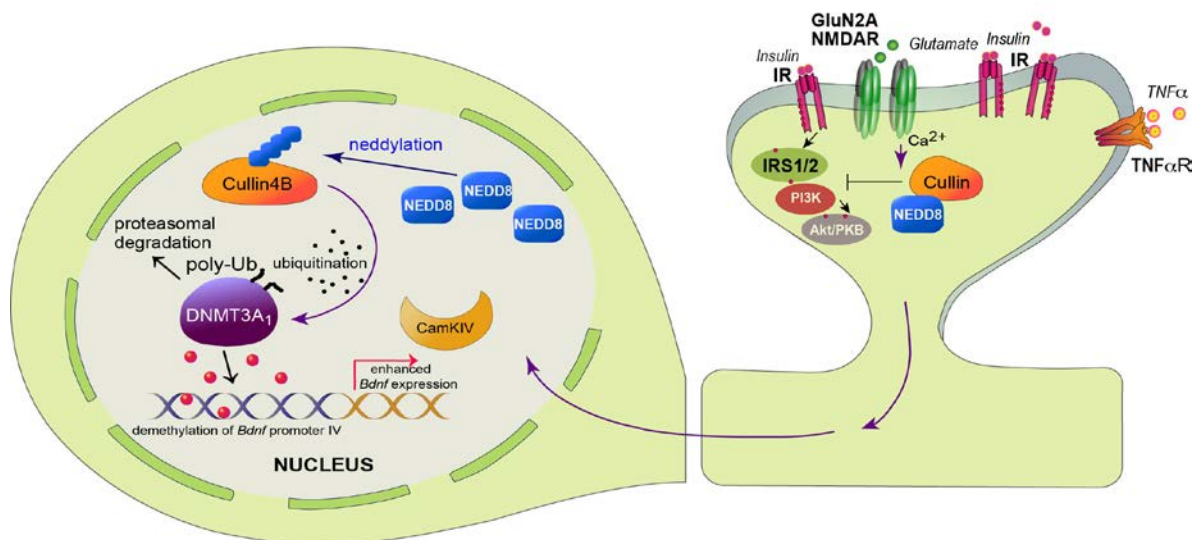

Figure 1: Overview: Interplay DNA-methylation and NMDAR signaling.

Although Nedd8 is less abundant at synaptic sites, we collected evidence for a synaptic role of neddylation. The so called metabolic syndrome (MetS) has become a major threat for healthy living itself and active aging. Recent studies suggest that cognitive decline induced by the metabolic syndrome is caused by impairments of insulin signaling in the hippocampus. Insulin and pro-inflammatory signaling are risk factors for synaptic function and, neglected frequently, amyloid load and synaptic insulin resistance increases the risk for Alzheimer's disease by a factor of 7-10. In ongoing work, we found that a degradation of the Insulin receptor scaffolds (IRS) is crucial for the induction of insulin resistance in the hippocampus. A proteomic screen performed in collaboration between the ISAS and the LIN revealed substantially elevated Cullin3 levels following the induction of synaptic insulin resistance. Intriguingly, we could block synaptic insulin resistance with a Nedd8 inhibitor (MLN4924), which is in clinical testing, and show that IRS1 and 2 are degraded in a neddylation-sensitive manner in insulin-resistant synapses. Meanwhile, several tools have been developed in our laboratories to study neddylation in the context of synaptic aging. We have assessed the topology of synaptic insulin signaling pathways and revealed the molecular basis of the interplay between synaptic insulin resistance and AD. To this end we have developed an animal model for a population of high-risk aging with MetS and increased amyloid load

3.2 Neuron-glia communication and contact rigidity: A molecular entry point to rejuvenate synaptic transmission?

Mounting evidence suggests that neuronal cells utilize not only electrical and chemical but also mechanical means for signalling and intercellular communication. This holds true particularly during development and the establishment of neural circuits but also in ageing. Mechanical forces regulate cellular properties in non-neuronal cells including morphology, differentiation, and gene expression. Adherent cells actively measure the stiffness of their surrounding and transduce this information via activation of intracellular signalling cascades. However, the impact and the mechanisms of mechanotransduction in the context of neuronal

cell biology haven't been addressed in much detail yet. This is surprising since the viscoelastic properties of the brain are remarkable. It clearly belongs to the softest tissues in the body, brain regions differ in their elastic modulus, and local stiffness gradients are crucial to guide axonal growth. Magnetic resonance elastography has shown that brain stiffness changes during development, most likely due to changed dynamics in cell adhesion molecules, extracellular matrix (ECM) proteins, and the cytoskeleton. As a consequence, changes in mechanical properties restricted to a cellular microdomain such as the rigidity of the ECM or cell-supporting substrates potentially have substantial impact on neuronal development. The effects of substrate stiffness on neurite formation are well documented and it was shown that primary neurons prefer softer substrates, thereby mimicking the native elastic properties during brain development. The process of neuronal differentiation is tightly coupled to synapse formation and indeed mechanochemical crosstalk is evident during synaptogenesis.

Various mechanosensors have been described including the recently discovered Piezo family. This class of mechanosensitive ion channel enables a rapid cellular response to changes in the mechanical properties of the surrounding since they exhibit one of the highest protein turnover rates. Numerous studies demonstrated that Piezo proteins play an essential role for cell migration and differentiation in the nervous system pointing to the importance of mechanotransduction during brain development and maturation. Hence, to investigate how general mechanical cues detected by mechanosensors can influence neuronal development, neuronal morphology, and, consequently, synaptic function we used polyacrylamide gels (PAA gels) that allow stiffness regulation via variation of acrylamide concentrations.

We found that neurons grown on soft substrates exhibit a higher density of synapses and presynaptic terminals, enhanced levels of synaptic proteins as well as an advanced developmental switch of the NMDA receptor subunit 2A to 2B in immature neurons, pointing towards an early onset of maturation. Moreover, the complexity of dendritic arborization was determined by substrate stiffness. Although neuronal maturation as evidenced by synapse density, are comparable between soft and stiff conditions at the end of maturation in culture, neuronal translation rates and dendritic arborisation remain enhanced in mature neurons on soft substrates. Mass spectrometry provided an inventory of accompanying relative changes in protein expression and largely confirmed the above results regarding accelerated maturation on softer substrates. Especially the upregulation of actin regulators on softer substrates are of interest as they might present links towards downstream signaling cascades in neuronal mechanosignalling. Notably, our dataset reveals the downregulation of proteins known to be expressed during early neuronal differentiation and neurite extension on soft substrate, advocating that compliant stiffness conditions favor maturation of neuronal networks. In summary, the stiffness-regulated proteomic inventory will provide a useful resource to pinpoint further molecular mechanotransduction mechanisms in the future.

Free intracellular calcium is central for signalling in biological systems and controls complex functions such as vesicle release, synaptic plasticity and gene transcription in neurons. Indeed, we found an increased synaptic vesicle recycling in neurons cultured on compliant gels. This effect can be related to the higher frequency of spontaneous $Ca2+$ oscillations on compliant gels suggesting that differences in Ca2 signaling might explain how substrates stiffness modulate neuronal development. We, therefore, concentrated on one class of key players in mechanotransduction, the stretch-gated cation channels, comprising channels from the TRP- and ENaC/Dec superfamily or the recently discovered Piezo family, all of them allowing increased cation flux when activated. $Ca2+$-imaging revealed that on compliant gels the number of calcium releases per second is significantly increased compared to stiffer substrates or glass coverslips in young cultures that was likely mediated by strong calcium influx through Piezo-1. This is supported by the elevated Piezo-1 expression on soft material in vitro as well as by the downregulation of Piezo-1 in vivo with age

Collectively, these findings add a new level of complexity to the crosstalk between mechanosensitive channels and tissue mechanics suggesting a feedback loop between sensed tissue stiffness and expression of stretch-gated ion channels. We propose that calcium- and mechanosignaling are coupled in particular in development and directy impact protein translation. Most important, the findings show that studies on neuronal development

and function using primary neurons should take the influence of mechanosignaling into account when choosing appropriate substrates for neuronal cultures. A paper describing these results is currently under consideration.

Neuronal Aging in a Dish

DIV 15   DIV 45   DIV 90

100 Pa = soft „young brain"
1000 Pa = intermediate
10T Pa = stiff „old brain"

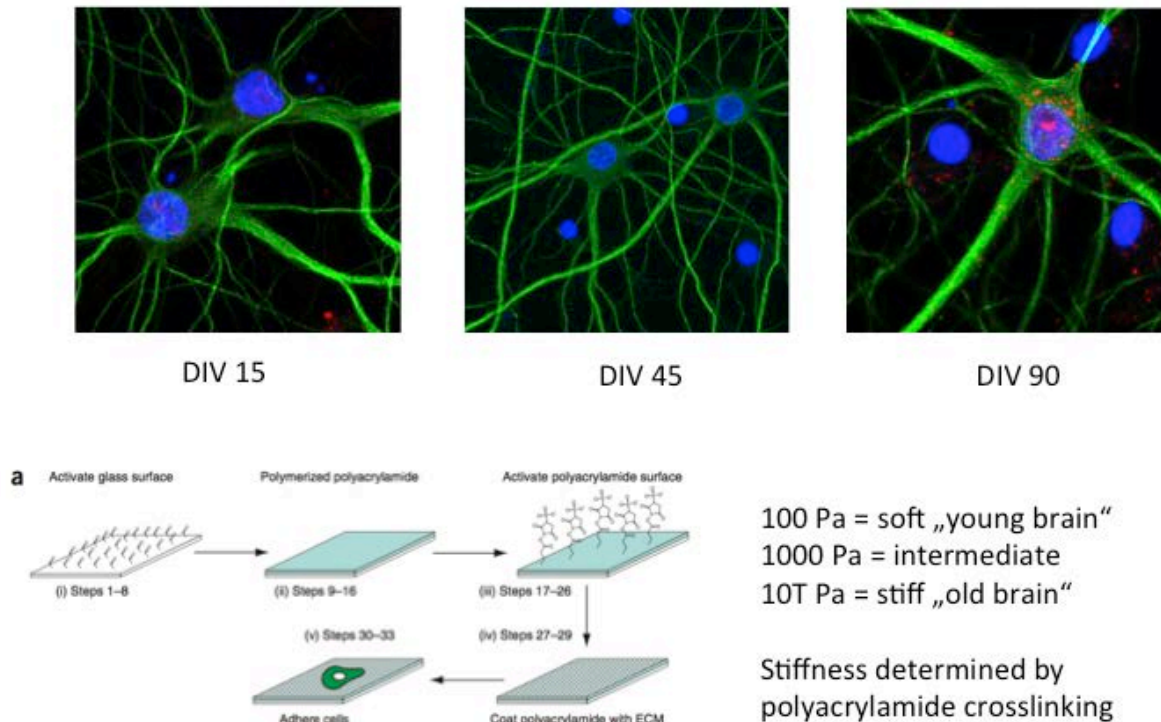Stiffness determined by polyacrylamide crosslinking

Figure 2 There is compelling evidence that neuronal primary cultures, which are the most accessible experimental system to study synaptic aging, exhibit dramatically accelerated aging (including distorted mitochondria, accumulation of reactive oxygen species in mitochondria, multilamellar vesicles, amyloidogenesis and proteasome impairment, as well as up-regulation of p53 and p21. We can manipulate aging by plating on surfaces with different stiffness that simulate the young and the old brain.

3.3 Rejuvenating intrinsic excitability in aging neurons

Changes in the expression or functional properties of ion channels at the level of the axon initial segment, where action potentials are initiated, or modifications in dendritic excitability, affecting the integration of synaptic inputs, can strongly influence the input-output function of a neuron rendering it more or less excitable (Dumenieu et al., 2017). In our studies we seeked to understand the contribution of low-voltage activated calcium channels and potassium channels to intrinsic excitability in mature granule cells of the dentate gyrus. Several studies have shown that it is relatively difficult to induce synaptic plasticity in mature but not in newborn dentate granule cells. Mature granule cells show also a strong dendritic attenuation of voltage signals, making the contribution of individual synaptic inputs to action potential initiation small. Therefore, non-synaptic forms of plasticity may play a more dominant role in this particular cell type than in other hippocampal neurons.

We recently investigated the role of T-type channels for bursting firing of mature granule cells (Dumenieu et al., 2018). Due to the particularly robust short-term facilitation properties of the mossy fibers-CA3 synapses, the pattern of action potentials that reaches the mossy fiber terminals strongly controls the probability that a given CA3 pyramidal cell will fire action potentials in response to this input. We found that T-type channels, specially the Cav3.2 subtype, are crucial for the bursting firing in mature granule cells *in vitro* (Dumenieu et al., 2018). In a set of *in vivo* experiments, it turned out that mice lacking the Cav3.2 channel

have an impaired ability to fire bursts of action potentials and that the transfer of information from the dentate-to-CA3 is also compromised in these animals. The data show that Cav3.2 channels are strong modulators of bursting and can be considered a critical molecular switch that enables effective information transfer from mature granule cells to the CA3 pyramids. This is a likely mechanism to be altered in aging.

3.4 Synaptic dysfunction in aging

Recent work from the consortium showed that posttranslational modification impact on the mechanism by which amyloid-β induces synaptic dysfunction (Grochowska et al., 2017). It is nowadays widely believed that accumulation of oligomeric non-fibrillar Amyloid-β (Aβo) disrupts normal synaptic function at early stages of Alzheimer's Disease (AD) and numerous studies suggest that soluble Aβo play a key role in the onset of synaptic dysfunction in AD. The molecular identity of Aβo that cause synapse loss and impair synaptic plasticity, however, is still unclear. Multiple posttranslationally modified Aβo peptides were reported in AD patients and among those the amino-terminally truncated, pyroglutamated form of Aβ (Aβ3(pE)-42) is abundant in AD brains and seeds highly toxic co-oligomers with conventional Aβ1-42. Aβ3(pE)-42 exhibit distinct structural features that might carry specific neurotoxic activity. A pressing matter is, whether modified Aβ species can induce synaptic dysfunction on their own and if so, whether they induce neuronal pathology by different means.

We found that posttranslationally modified Aβ3(pE)-42 can induce neuronal dysfunction on its own without co-oligomerization with conventional Aβ1-42. One influential view of the molecular mechanism that underlies synaptic dysfunction induced by conventional Aβ1-42 focuses on an interruption of NMDAR and mGluR5-modulated synaptic signaling. These effects are likely mediated through an association of Aβ with the Prion protein. Pathological signaling of full-length Aβ1-42 that induces synaptic dysfunction depends upon a functional and most likely also physical interaction of NMDAR, Prp and mGluR5 and we found evidence that this is indeed the case. However, we realized that in stark contrast to conventional Aβ1-42, Aβ3(pE)-42 did not directly associate with synaptic membranes or the Prion protein. Moreover, Aβ3(pE)-42 induced synaptic dysfunction is not related to NMDAR signaling and Aβ3(pE)-42 induced impairments of neuronal plasticity cannot be rescued by dopamine D1-receptor agonists. Thus, fundamental differences exist in pathological signaling. Aβ3(pE)-42 is readily taken up by astrocytes and potently induces glial release of the pro-inflammatory cytokine TNFα. With several experiments we could show that pathological signaling of Aβ3(pE)-42 oligomers operate via TNF receptors in neurons whereas Aβ1-42 oligomers operate via PrP-mGluR5-NMDAR. Collectively the data fit to a scenario where neuroinflammatory processes together with direct synaptotoxic effects are caused by soluble oligomeric Aβ and contribute synergistically to the onset of AD. It is therefore questionable that targeting a synaptic Aβ-receptor will fully revert synaptic dysfunction in AD.
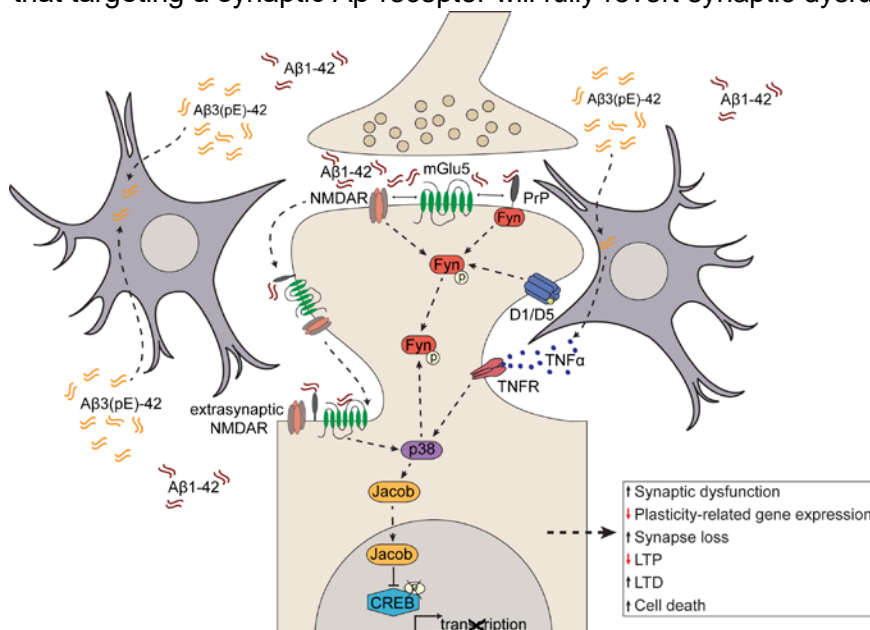

Figure 3: Our work highlights the possibility that posttranslationally modified Aβ oligomers might trigger synaptic dysfunction via different pathological signalling pathways. It is possible that there is a considerable variability in the levels of modified Aβ isoforms between AD patients, resulting in different pathomechanisms, and we speculate that this aspect could lead to

different clinical trajectories.

4. General Output of the Project

4.1 Publications

1. Bayraktar G, Kreutz MR. The Role of Activity-Dependent DNA Demethylation in the Adult Brain and in Neurological Disorders. Front Mol Neurosci. 2018 May 23;11:169.

2. Yun D, Zhuang Y, Kreutz MR, Behnisch T. The role of 19S proteasome associated deubiquitinases in activity-dependent hippocampal synaptic plasticity. Neuropharmacology. 2018 May 1;133:354-365.

3. Bayraktar G, Kreutz MR. Neuronal DNA Methyltransferases: Epigenetic Mediators between Synaptic Activity and Gene Expression? Neuroscientist. 2018 Apr;24(2):171-185.

4. Mikhaylova M, Bär J, van Bommel B, Schätzle P, YuanXiang P, Raman R, Hradsky J, Konietzny A, Loktionov EY, Reddy PP, Lopez-Rojas J, Spilker C, Kobler O, Raza SA, Stork O, Hoogenraad CC, Kreutz MR. Caldendrin Directly Couples Postsynaptic Calcium Signals to Actin Remodeling in Dendritic Spines. Neuron. 2018 Mar 7;97(5):1110-1125.e14.

5. Homiack D, O'Cinneide E, Hajmurad S, Barrileaux B, Stanley M, Kreutz MR, Schrader LA. Predator odor evokes sex-independent stress responses in male and female Wistar rats and reduces phosphorylation of cyclic-adenosine monophosphate response element binding protein in the male, but not the female hippocampus. Hippocampus. 2017 Sep;27(9):1016-1029.

6. Zhao B, Meka DP, Scharrenberg R, König T, Schwanke B, Kobler O, Windhorst S, Kreutz MR, Mikhaylova M, Calderon de Anda F. Microtubules Modulate F-actin Dynamics during Neuronal Polarization. Sci Rep. 2017 Aug 29;7(1):9583.

7. Grochowska KM, Yuanxiang P, Bär J, Raman R, Brugal G, Sahu G, Schweizer M, Bikbaev A, Schilling S, Demuth HU, Kreutz MR. Posttranslational modification impact on the mechanism by which amyloid-β induces synaptic dysfunction. EMBO Rep. 2017 Jun;18(6):962-981.

8. Kiran U, Regur P, Kreutz MR, Sharma Y, Chakraborty A. Intermotif Communication Induces Hierarchical Ca(2+) Filling of Caldendrin. Biochemistry. 2017 May 16;56(19):2467-2476.

9. Duménieu M, Oulé M, Kreutz MR, Lopez-Rojas J. The Segregated Expression of Voltage-Gated Potassium and Sodium Channels in Neuronal Membranes: Functional Implications and Regulatory Mechanisms. Front Cell Neurosci. 2017 Apr 24;11:115.

10. Spilker C, Grochowska KM, Kreutz MR. What do we learn from the murine Jacob/Nsmf gene knockout for human disease? Rare Dis. 2016 Sep 30;4(1):e1241361.

11. Hrdinka M, Sudan K, Just S, Drobek A, Stepanek O, Schlüter D, Reinhold D, Jordan BA, Gintschel P, Schraven B, Kreutz MR. Normal Development and Function of T Cells in Proline Rich 7 (Prr7) Deficient Mice. PLoS One. 2016 Sep 22;11(9):e0162863.

12. Kravchick DO, Karpova A, Hrdinka M, Lopez-Rojas J, Iacobas S, Carbonell AU, Iacobas DA, Kreutz MR, Jordan BA. Synaptonuclear messenger PRR7 inhibits c-Jun ubiquitination and regulates NMDA-mediated excitotoxicity. EMBO J. 2016 Sep 1;35(17):1923-34.

13. Melgarejo da Rosa M, Yuanxiang P, Brambilla R, Kreutz MR, Karpova A. Synaptic GluN2B/CaMKII-α Signaling Induces Synapto-Nuclear Transport of ERK and Jacob. Front Mol Neurosci. 2016 Aug 10;9:66.

14. Bera S, Raghuram V, Mikhaylova M, Kreutz MR. A plasmid-based expression system to study protein-protein interactions at the Golgi in vivo. Anal Biochem. 2016 Jun 1;502:50-52.

15. Lopez-Rojas J, Kreutz MR. Mature granule cells of the dentate gyrus--Passive bystanders or principal performers in hippocampal function? Neurosci Biobehav Rev. 2016 May;64:167-74.

16. Heise C, Schroeder JC, Schoen M, Halbedl S, Reim D, Woelfle S, Kreutz MR, Schmeisser MJ, Boeckers TM. Selective Localization of Shanks to VGLUT1-Positive Excitatory Synapses in the Mouse Hippocampus. Front Cell Neurosci. 2016 Apr 26;10:106.

17. Yuan Xiang P, Janc O, Grochowska KM, Kreutz MR, Reymann KG. Dopamine agonists rescue Aβ-induced LTP impairment by Src-family tyrosine kinases. Neurobiol Aging. 2016 ;40:98-102.

18. Dinamarca MC, Guzzetti F, Karpova A, Lim D, Mitro N, Musardo S, Mellone M, Marcello E, Stanic J, Samaddar T, Burguière A, Caldarelli A, Genazzani AA, Perroy J, Fagni L, Canonico PL, Kreutz MR, Gardoni F, Di Luca M. Ring finger protein 10 is a novel synaptonuclear messenger encoding activation of NMDA receptors in hippocampus. Elife. 2016 Mar 15;5:e12430.

19. Spilker C, Nullmeier S, Grochowska KM, Schumacher A, Butnaru I, Macharadze T, Gomes GM, Yuanxiang P, Bayraktar G, Rodenstein C, Geiseler C, Kolodziej A, Lopez-Rojas J, Montag D, Angenstein F, Bär J, D'Hanis W, Roskoden T, Mikhaylova M, Budinger E, Ohl FW, Stork O, Zenclussen AC, Karpova A, Schwegler H, Kreutz MR. A Jacob/Nsmf Gene Knockout Results in Hippocampal Dysplasia and Impaired BDNF Signaling in Dendritogenesis. PLoS Genet. 2016 Mar 15;12(3):e1005907.

20. Lopez-Rojas J, Heine M, Kreutz MR. Plasticity of intrinsic excitability in mature granule cells of the dentate gyrus. Sci Rep. 2016 Feb 9;6:21615.

21. Dieterich DC, Kreutz MR. Proteomics of the Synapse--A Quantitative Approach to Neuronal Plasticity. Mol Cell Proteomics. 2016 Feb;15(2):368-81.

22. Mikhaylova M, Bera S, Kobler O, Frischknecht R, Kreutz MR. A Dendritic Golgi Satellite between ERGIC and Retromer. Cell Rep. 2016 Jan 12;14(2):189-99.

23. Hradsky J, Bernstein HG, Marunde M, Mikhaylova M, Kreutz MR. Alternative splicing, expression and cellular localization of Calneuron-1 in the rat and human brain. J Histochem Cytochem. 2015 Oct;63(10):793-804.

24. Lever MB, Karpova A, Kreutz MR. An Importin Code in neuronal transport from synapse-to-nucleus? Front Mol Neurosci. 2015 Jul 21;8:33.

25. Neumann JR, Dash-Wagh S, Jüngling K, Tsai T, Meschkat M, Räk A, Schönfelder S, Riedel C, Hamad MI, Wiese S, Pape HC, Gottmann K, Kreutz MR, Wahle P. Y-P30 promotes axonal growth by stabilizing growth cones. Brain Struct Funct. 2015 Jul;220(4):1935-50.

26. Panayotis N, Karpova A, Kreutz MR, Fainzilber M. Macromolecular transport in synapse to nucleus communication. Trends Neurosci. 2015 Feb;38(2):108-16.

27. Navarro G, Aguinaga D, Moreno E, Hradsky J, Reddy PP, Cortés A, Mallol J, Casadó V, Mikhaylova M, Kreutz MR, Lluís C, Canela EI, McCormick PJ, Ferré S. Intracellular calcium levels determine differential modulation of allosteric interactions within G protein-coupled receptor heteromers. Chem Biol. 2014 Nov 20;21(11):1546-56.

28. Rosenberg T, Gal-Ben-Ari S, Dieterich DC, Kreutz MR, Ziv NE, Gundelfinger ED, Rosenblum K. The roles of protein expression in synaptic plasticity and memory consolidation. Front Mol Neurosci. 2014 Nov 12;7:86.

29. Kaushik R, Grochowska KM, Butnaru I, Kreutz MR. Protein trafficking from synapse to nucleus in control of activity-dependent gene expression. Neuroscience. 2014 Nov 7;280:340-50.

30. Yuanxiang P, Bera S, Karpova A, Kreutz MR, Mikhaylova M. Isolation of CA1 nuclear enriched fractions from hippocampal slices to study activity-dependent nuclear import of synapto-nuclear messenger proteins. J Vis Exp. 2014 Aug 10;(90):e51310.

31. Reddy PP, Raghuram V, Hradsky J, Spilker C, Chakraborty A, Sharma Y, Mikhaylova M, Kreutz MR. Molecular dynamics of the neuronal EF-hand Ca2+-sensor Caldendrin. PLoS One. 2014 Jul 24;9(7):e103186.

32. Mikhaylova M, Schumacher A, Borutzki C, Neumann JR, Macharadze T, El-Mousleh T, Wahle P, Zenclussen AC, Kreutz MR. Analysis of Y-P30/Dermcidin expression and properties of the Y-P30 peptide. BMC Res Notes. 2014 Jun 26;7:400.

33. Gomes GM, Dalmolin GD, Bär J, Karpova A, Mello CF, Kreutz MR, Rubin MA. Inhibition of the polyamine system counteracts β-amyloid peptide-induced memory impairment in mice: involvement of extrasynaptic NMDA receptors. PLoS One. 2014 Jun 12;9(6):e99184.

34. Mikhaylova M, Karpova A, Bär J, Bethge P, YuanXiang P, Chen Y, Zuschratter W, Behnisch T, Kreutz MR. Cellular distribution of the NMDA-receptor activated synapto-nuclear messenger Jacob in the rat brain. Brain Struct Funct. 2014 May;219(3):843-60.

35. Landgraf P, Mikhaylova M, Macharadze T, Borutzki C, Zenclussen AC, Wahle P, Kreutz MR. Binding of Y-P30 to syndecan 2/3 regulates the nuclear localization of CASK. PLoS One. 2014 Feb 3;9(2):e85924.

36. Grabrucker S, Jannetti L, Eckert M, Gaub S, Chhabra R, Pfaender S, Mangus K, Reddy PP, Rankovic V, Schmeisser MJ, Kreutz MR, Ehret G, Boeckers TM, Grabrucker AM. Zinc deficiency dysregulates the synaptic ProSAP/Shank scaffold and might contribute to autism spectrum disorders. Brain. 2014 Jan;137(Pt 1):137-52.

37. Bera S, Kreutz MR. When synaptic proteins meet the genome: transcriptional regulation in cell death and plasticity by the synapto-nuclear messenger Jacob. Neuropsychopharmacology. 2014 Jan;39(1):245-6.

4.2 Scientific Meetings organized by Network Members

The Healthy Ageing Symposium 2017 was held at the LIN in Magdeburg.

Michael Kreutz (top) and Kasia Grochowska from his group (bottom) give talks at the symposium.

4.3 Transfer activities

Activity	Comment
Public Outreach	Presentations at the annual Long Night of Science with 1600 visitors
Press releases	Press releases were launched to local newspapers

4.4 Thesis

In total 8 PhD theses and 2 MSc theses were completed during the course of the studies.

5. Sustainability of Structures / Cooperation and Outlook

To tackle these problems a number of institutes of the Leibniz Association founded the *Leibniz-Forschungsverbund Gesundes Altern* **(Leibniz Research Network on Healthy Aging)** in 2012. The major aim of this consortium is to understand the biological and socioeconomical mechanisms of aging and to develop intervention strategies. Here, three Leibniz Institutes, the FLI in Jena (Fritz Lipmann Institute, Age Research), the FMP in Berlin (Molecular Pharmacology) and the LIN in Magdeburg (Neurobiology) applied for network projects aiming to improve our understanding of molecular causes of aging in key areas. Within the '**Leibniz Research Network on Healthy Aging'** members of the consortium established a Focus Group 'Synaptic ageing: Implications for the ageing brain'. Michael R. Kreutz is head of this Focus Group. The Focus Group will try to join forces in order to attract extramural funding with the ultimate goal to apply for a DFG-Forschungsgruppe. Finally,

several principal investigators of the consortium played a significant a role to establish a DFG-funded GRK on synaptic ageing called SynAGE in Magdeburg.