Sachbericht

„Auf der Suche nach dem Engramm: Die Integration synaptischer Signale im Epigenom von Nervenzellen"

Final report

In search of the engram: The integration of synaptic signals in the epigenome of neurons

Table of content

Executive summary

In this project we aimed to investigate how an engram is stored at the cellular level. We followed up on the hypothesis that signaling pathways from post- and presynapses of the same neuron converge in the nucleus and induce long-lasting alterations in gene expression that in turn feed-back to synaptic function and thereby impact on the formation of long-term memory. To this end we performed genome-wide DNA methylation analysis in selected cell populations involved in memory formation, identified novel synapse-to-nucleus signaling mechanisms and elucidated the nuclear function of protein transport from synapse-to-nucleus. To this end we employed a multidisciplinary approach that combined live-imaging in neurons, epigenetics, transcriptomics, proteomics, molecular cell biology methodology with studies ranging from individual molecules to whole animal experimentation. In several high-ranking publications we developed a novel view on synapse-to-nucleus communication. According to this view rather specific signals from the pre- and postsynapse induce nucleocytoplasmic shuttling of proteins and even active retrograde transport of synapto-nuclear messengers to the nucleus. We believe that proteins that directly interact with NMDAR subunits can be transported to the nucleus. Following nuclear import these proteins associate with transcription factor complexes and can induce sustained changes in gene expression. This mechanism allows for encoding of signals at the site of origin and decoding in the nucleus. Further work deciphered how signals from the pre- and postsynapse might interact to elicit in integrative genomic response to changes in synaptic activity. Finally, we could establish mechanisms how synaptic signals as well as learning and memory control and affect DNA-methylation, which in turn indicates that the memory trace is more dispersed and that epigenetic mechanisms are part of the engram.

State of the art and aims of the project

Activity-induced changes in gene expression are a key mechanism underlying the conversion of rapid but otherwise transient usage-dependent modification of synaptic strength into persistent changes within engram cells. NMDAR signaling from synapse to nucleus regulates gene expression and is supposed to play a crucial role in synaptic plasticity and long-term memory. In the past decade several studies have proposed mechanisms of activity dependent transport of synaptic proteins to the nucleus (Kaushik et al., 2014; Panayotis et al., 2015). This type of signaling is conceptually appealing because it allows for local encoding of signals at the site of origin and decoding in the nucleus (Jordan and Kreutz, 2009; Karpova et al., 2012).

Neurons are polarized cells with an elaborate complex dendritic tree and an axon that can bridge vast distances particularly in the human nervous system. Long distance signaling is critical for maintenance of synaptic contacts but the integration of signaling events at remote synaptic sites into a nuclear response that stabilizes cellular networks and associated functions is not well understood. Important aspects in this regard are the antero- and retrograde transport of cargo to dendrites and axons and the active nuclear import of protein messengers in response to synaptic activity (Jordan & Kreutz, 2009; Fainzilber et al., 2011). NMDAR are tethered to scaffolding proteins and a larger signaling complex is assembled. In the past four years we found that this complex is a rich source of synapto-nuclear protein messenger. In total five of these proteins (Jacob, Abi-1, RNF10, Prr7 and Rack1) were identified by us and our collaborators in various screens and the work done on these proteins has led to the concept that different NMDAR signals induce the nuclear translocation of different proteins.

In our previous work we described synaptic localization of CtBP1, which was studied before as transcriptional regulator and chromatin modulator in context of gene expression regulation in non-neuronal cells (tom Dieck et al., 2005; Hubler et al., 2012). This project hypothesized that CtBP1 could shuttle between synapses and nuclei and transduce information about levels of synaptic activity into regulation of gene expression and therefore contribute to usage-dependent neuronal plasticity. We planned to investigate the shuttling of CtBP1 in neurons and explore the mechanism controlling the process and its impact on regulation of neuronal plasticity-related genes.

Moreover, with this project we wanted to clarify how synaptic signals from pre- and postsynapse are transduced to the nucleus, how they regulate gene expression and how alterations in gene expression feed back to synaptic function. We wanted to investigate whether plasticity-relevant signals will induce nuclear import of synapto-nuclear protein messenger (SPNM) and elucidate the composition of the macromolecular transport complexes. Finally, we tried to understand how signals how signals from the pre- and postsynapse might interact to elicit in integrative genomic response to changes in synaptic activity.

Development of project, deviation form original research program, difficulties in conducting the proposed research

All in all, the research work was conducted in very good alignment with the proposed workflow. We learned about mechanisms of transport, the composition of transport complexes and the molecular identity of SPNM. The hypothesis about Piccolo and Bassoon-dependent synaptic localization of CtBP1 was confirmed and complex regulation of this recruitment was dissected. The role of synapto-nuclear shuttling of CtBP1 and expressional control by CtBP1 in activity-induced regulation of neuroplasticity-relevant genes was addressed.

Results of the project, their significance and implementation

In recent years signalling from synapse-to-nucleus has gained increasing attention and the hypothesis that nuclear gene expression is controlled by a fast Ca2+-mediated component and a slower component involving synapto-nuclear protein import has received support from several lines of evidence (Jordan & Kreutz, 2009; Karpova et al., 2012; Kaushik et al., 2014; Panayotis et al., 2015). For the sake of space we want to show-cast this principle by briefly summarizing our recent work supported by the Leibniz Foundation on Jacob, RNF10, PRR7 as well as DNMT3A1.
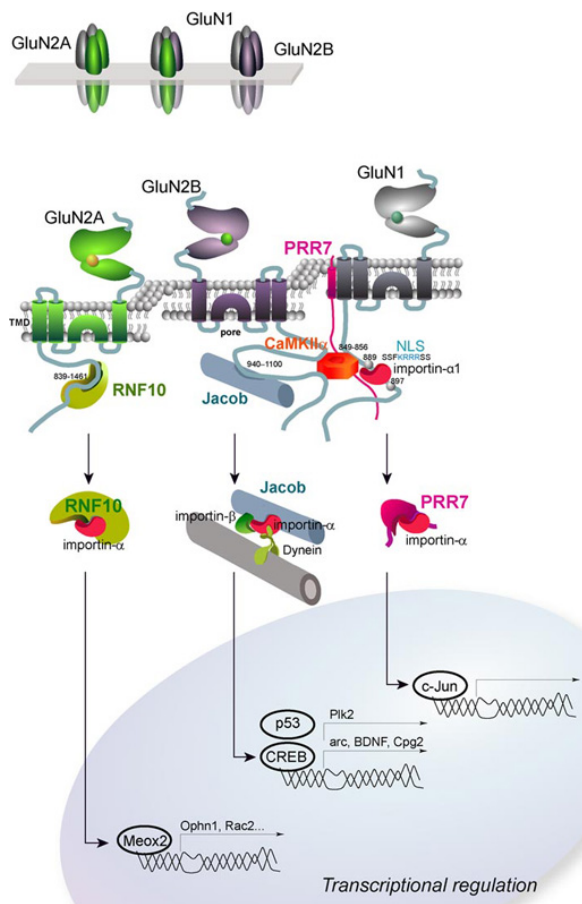

Figure 1: NMDAR-to-nucleus signaling. Taken together our work contributed to a novel concept of synapse-to-nucleus communication. We believe that proteins that directly interact with NMDAR subunits can be transported to the nucleus. Following nuclear import these proteins associate with transcription factor complexes and can induce sustained changes in gene expression. This mechanism allows for encoding of signals at the site of origin and decoding in the nucleus. We think that nuclear import of these proteins can keep the nucleus informed about the number of newly inserted NMDAR, their synaptic/extrasynaptic localization, the NMDAR subtype, NMDAR-dependent LTP/LTD and so on.

Jacob is a protein that directly associates with the GluN2B subunit of NMDAR (see also De la Rosa et al. 2016 for the mechanism of dissociation) and protein transport of Jacob to the nucleus couples NMDAR activity to gene transcription. Long-distance shuttling of Jacob encodes and transduces the synaptic or extrasynaptic origin of NMDAR signals to the nucleus and we could show that Jacob operates as a mobile hub that docks an NMDAR-derived signalosome to nuclear target sites like CREB, p53 and others (Karpova et al., 2013). A constitutive gene knock-out where Jacob is already absent during neuronal development results in hippocampal dysplasia. The mice exhibit shorter dendrites and less arborization in hippocampal pyramidal neurons in CA1, a lower number of dendritic spines, enlarged TIMM staining of the DG/CA3 projection, impaired LTP and contextual fear conditioning, an increased dopaminergic innervation, lower CREB and Ser133 pCREB levels as well as impaired BDNF-signaling during dendritogenesis. Most important during early development BDNF induces the nuclear translocation of Jacob in an NMDAR-dependent manner, which results in increased phosphorylation of CREB and enhanced CREB-dependent BDNF gene transcription. Thus, the lack of Jacob interrupts a positive feedback loop between BDNF-signalling, subsequent nuclear import of Jacob, activation of CREB and enhanced BDNF gene transcription, which in turn promotes dendritogenesis and synapse formation (Spilker et al., 2016).

Like Jacob RNF10 and Prr7 harbor a NLS and nuclear trafficking following NMDAR activation requires binding of importin-α to this NLS. RNF-10 was identified in a YTH screen with the C-terminal tail of GluN2A. RING finger protein family members are involved in transcriptional regulation and RNF10 interacts with the transcription factor Meox2. We could show that

Meox2/RNF10 target genes include several genes involved in excitatory synaptic transmission like oligophrenin-1, a Rho-GTPase, where mutations in the oligophrenin gene have been associated with mental retardation. We found that RNF10 is indeed present at synapses and in the nucleus of pyramidal neurons and we could confirm that RNF10 accumulates in the nucleus following long-distance protein transport. In contrast to Jacob that directly binds to the C-terminal tail of GluN2B RNF10 directly interacts with GluN2A and most important the nuclear import of RNF10 can be blocked with a GluN2A antagonist but not with an antagonist of GluN2B receptors, which is again the opposite of what we found with Jacob. Taken together our data indicate that RNF10 represents a novel synapto-nuclear protein messenger responsible for the transduction of GluN2A receptor signals and that RNF10 silencing prevents the maintenance of LTP as well as LTP-dependent structural modifications of dendritic spines (Dinarmaca et al., 2016).

Prr7 is a transmembrane adaptor protein (Trap) and this protein family is well known in T-cell biology because they assemble a membrane poximal signalosome that is essential for TCR activation. In brain it is part of the NMDAR/PSD95 complex, it binds directly with a PDZ binding motif to the 3rd PDZ domain of PSD-95 and we also found a cis interaction of the first 44 amino acids of Prr7 that include the extracellular part, the TMD and a short stretch of the juxtamembranous region with the GluN1 subunit of NMDAR. This finding then raised the question whether Prr7 might be an auxilliary subunit of GluN1. But we found so far no effect of PRR7 overexpression, knockdown, gene knockout on either trafficking, surface expression and also NMDAR channel properties. Instead it turned out that Prr7 strictly upon NMDAR activation translocate to the nucleus. In the nucleus Prr7 associated with the c-Jun complex and inhibits two key E3-ubiquitin ligases for ubiquitylation of c-Jun, Itch and FBW7. This mechanism is involved in NMDA-excitotoxicity (Kravchick et al., 2016).

Aberrant DNA methylation has been implicated in a plethora of studies in neuropsychiatric diseases including schizophrenia, bipolar and major depression disorders. It is widely believed that rapid and reversible DNA methylation is essential for stability of long-term memory but still very little is known how synaptic signals can induce changes in DNA-methylation to elicit enduring alterations in plasticity-related gene expression. N-methyl-D-aspartate receptor (NMDAR) signalling is instrumental in this regard and is also altered in schizophrenia as well as other neuropsychiatric disorders, and NMDAR antagonists are capable to induce psychotomimetic effects in healthy volunteers. However, a mechanistic link between NMDAR signalling and DNA-methylation is currently missing.

In recent work we show that activation of synaptic GluN2A-containing N-Methly-D-Aspartate-receptors drives the neddylation-dependent proteasomal degradation of the principal de novo DNA-methyltransfearse in brain, DNMT3A1. Collectively our data point to a mechanism that allows for the synaptic control of nuclear DNMT3A1 protein levels and that thereby creates a time window for reduced de novo DNA-methylation at a subset of target genes. In brief, we show-cast how long-distance signalling from NMDAR control nuclear protein levels of Dnmt3a1 via neddylation of the E3-ubiquitin ligase Cullin-4B, which in turn ubiquitylates Dnmt3a1, and this then results in hypomethylation of the bdnfIV promoter, increased BDNF expression and promotes late long-term potentiation (LTP). We found that nuclear DNMT3A1 protein levels in CA1 neurons are reduced following the induction of NMDAR-dependent LTP and most importantly following novel location learning. The time window for this downregulation (3-6hrs following NMDA-receptor stimulation - the protein synthesis and protein degradation sensitive phase of memory consolidation) is commensurate for a function of DNMT3A1 degradation in regulation of gene expression that is involved for long-term memory stabilization. Intriguingly, we found that novel location learning is neddylation-sensitive. Neddylation has not been investigated in any detail in neurons yet and only few reports have been published so far. Nedd8 is most abundant in neuronal nuclei and it is

tempting to speculate that activity-dependent neddylation might reduce protein levels not only of DNMT33A1 but also of other nuclear epigenetic modifiers (See Figure 2 below).
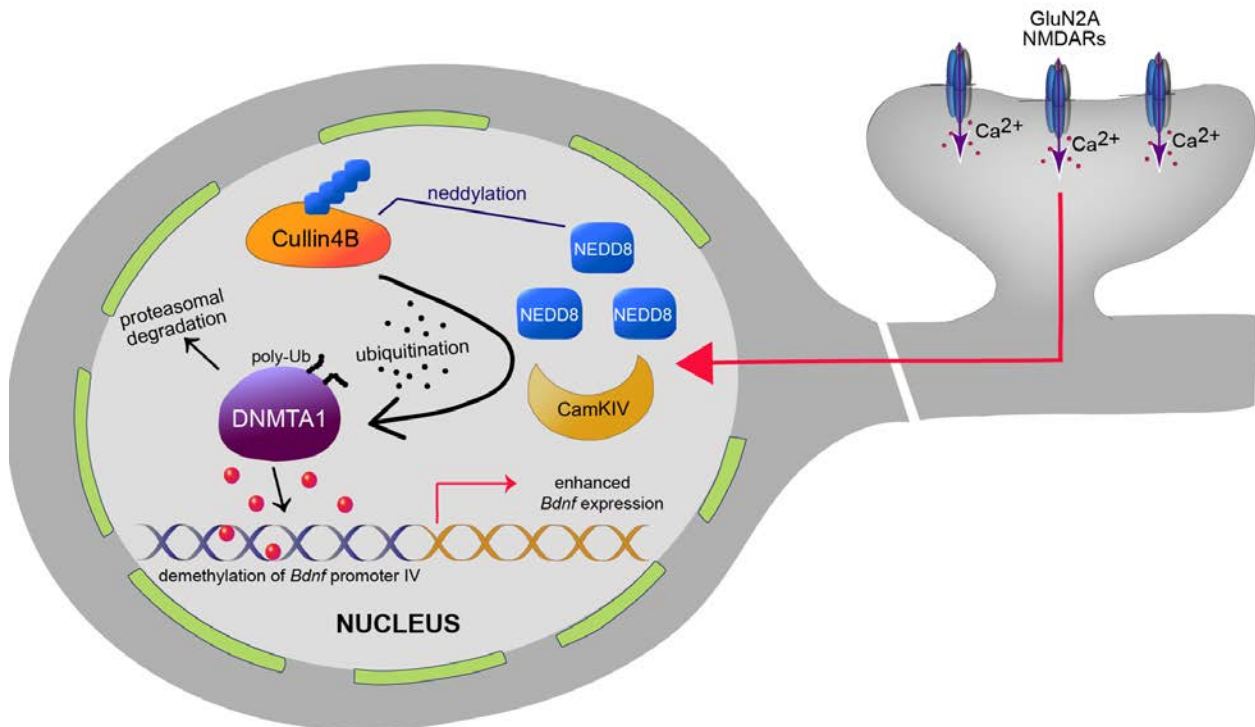
In further work we found that histone modifications predominantly changed during memory acquisition and correlated surprisingly little with changes in gene expression. Although long-lasting changes were almost exclusive to neurons, learning-related histone modification and DNA methylation changes also occurred in non-neuronal cell types, suggesting a functional role for non-neuronal cells in epigenetic learning (Halder et al., 2016). Finally, our data provide evidence for a molecular framework of memory acquisition and maintenance, wherein DNA methylation could alter the expression and splicing of genes involved in functional plasticity and synaptic wiring (Halder et al., 2016).

Finally, we have identified a new signaling pathway that controls neuronal gene expression in dependence on neuronal activity and contributes to this process. The transcriptional corepressor C-terminal binding protein 1 (CtBP1), which has been studied extensively in non-neuronal context by others, plays a central role in this signaling. In neurons, CtBP1 shows an intriguing dual localization to nuclei and to presynapses and shuttles between these two compartments dependently on levels of neuronal activity (Hübler et al., 2012; Ivanova et al., 2015). The publication in the EMBO Journal that is one of main outcomes of this sub-project threw light on mechanisms underlying the signaling through CtBP1. It describes that CtBP1 is attached at presynaptic active zone via direct biochemical interactions with presynaptic scaffolds Bassoon and Piccolo. The binding of CtBP1 to its presynaptic anchors is sensitive to changes in cellular NAD/NADH levels that are in turn altered dependently of levels of neuronal activity (Ivanova et al., 2015; Ivanova et al., 2016). Once the neuronal activity drops, CtBP1 is released form presynapse it shuttles to nucleus, where it contributes to reconfiguration of expressional patterns by gene

7

repression and chromaffin modification. This pathway can also explain the mechanism, by which CtBP1 contributes to the effect of ketogenic diet used widely for management of epilepsy (see
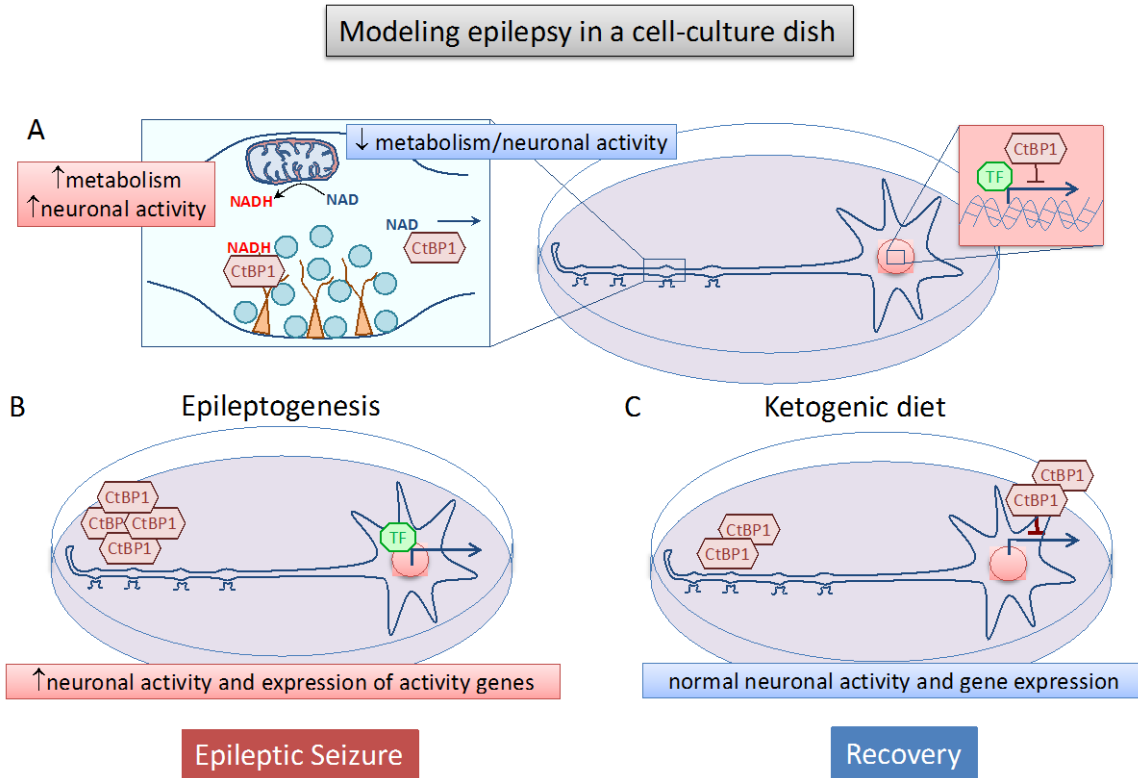

Figure 3 below).

Discovery of this novel signaling pathway that links regulation of neuronal activity-controlled gene expression with cellular metabolism was honored by the Hugo Junkers Prize for Research and Innovation by the State of Saxony-Anhalt in December 2015. Currently, we carry on with investigations of the cellular pathways controlling CtBP1presynapse-to-nucleus signaling and of the role of this pathway in function of normal circuit and in disease.

During the course of the studies we could confirm that both pathways might impact on the same promoters like the BDNF promoter (see below). However, it seems that conditions that drive for instance Jacob into the nucleus results in a synaptic localization of CtBP1. In other terms we found evidence for negative co-operativity.
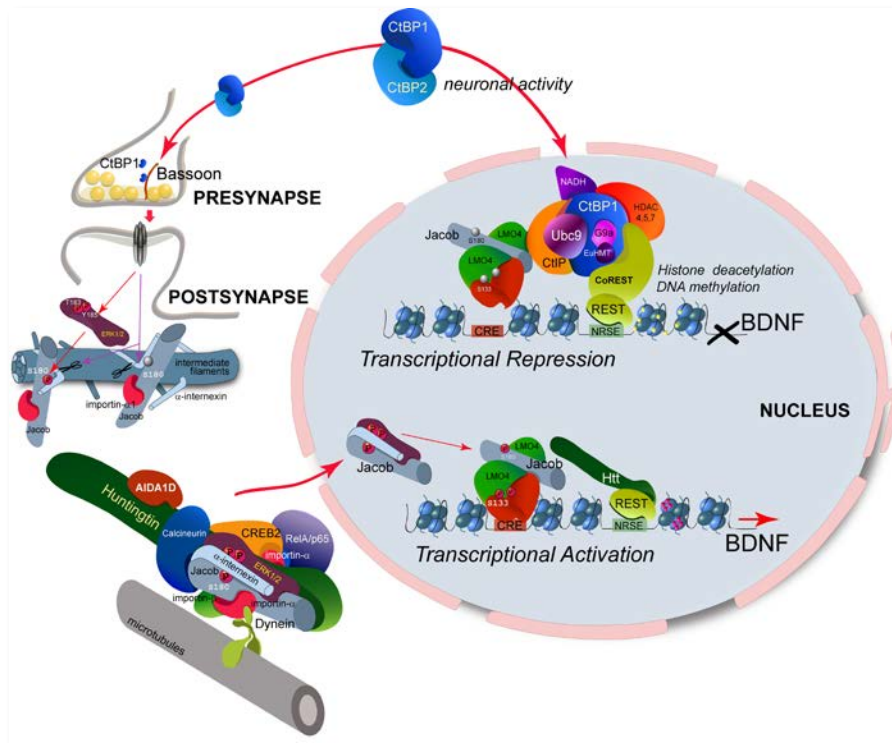

Figure 3: The communication of pre- and postsynapse with the nucleus via SPNM is exemplified at the BDNF-promoter. Different signals will induce transport of macromolecular complexes and control the activity of the promoter most likely via negative co-operativity.

Economical utilization of the obtained results

Results can be not directly economically applied; however, they build a solid basis for further preclinical investigations of role of the pathway in brain diseases and therapeutically approaches.

Co-operations

- Bryen A. Jordan, Albert Einstein College of Medicine, New York, USA
- Monica Di Luca & Fabrizio Gardoni, University of Milano, Italy
- Mike Fainzilber, Weizmann Institute, Rehovot, Israel
- Martin Zenker, Institute of Human Genetics, OVGU, Magdeburg
- M. Walter, Psychiatry and Psychotherapy, University Hospital Tubingen

PhD theses completed during the course of the studies

2013   Parameshwar Pasham Reddy   Dr. rer. nat. at OVGU Magdeburg
2014   Sujoy Bera   Dr. rer. nat. (summa cum laude) OVGU Magdeburg
2014   Rahul Kaushik   Dr. rer. nat. (summa cum laude) OVGU Magdeburg
2016   Julia Bär   Dr. rer. nat. (summa cum laude) OVGU Magdeburg
2016   Johannes Hradsky   Dr. rer. nat. (summa cum laude) OVGU Magdeburg
2015   Daniela Ivanova   Dr. Sc. nat. (summa cum laude) at OVGU Magdeburg
2017   Anika Dirks   Dr. sc. nat. OVGU Magdeburg
2017   Santosh Pothula   Dr. sc.nat. OVGU, Magdeburg

List of publications

- 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 pii: e201643519.
- 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 11(9):e0162863.
- 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 excitotoxity. EMBO J. 2016 Jul 25. pii: e201593070.
- 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 5. pii: e12430. *shared correspondence F1000Prime RECOMMENDED **1***
- Spilker C, Nullmeier S, Grochowska KM, Schumacher A, Butnara I, Macharadze T, Yuanxiang P, Gomes GM, Bayraktar G, Rodenstein C, Kolodziej A, Montag D, Angenstein F, Bär J, D'Hanis W, Roskoden T, Mikhaylova M, Budinger E, Ohl FW, Stork O, Karpova A, Zenclussen AC, Schwegler H, Kreutz MR. A jacob/nsmf gene knock-out results in hippocampal dysplasia and impaired BDNF signalling in dendritogenesis. PLoS Genet. 2016 12(3):e1005907.
- Melgarejo da Rosa M, Yuanxiang P, Brambilla R, Kreutz MR, Karpova A (2016) Synaptic GluN2B/CaMKII-Signaling Induces Synapto-Nuclear Transport of ERK and Jacob. Front. Mol. Neurosci. 2016 9:66. doi: 10.3389/fnmol.2016.00066.
- Halder R, Hennion M, Vidal RO, Shomroni O, Rahman RU, Rajput A, Centeno TP, van Bebber F, Capece V, Garcia Vizcaino JC, Schuetz AL, Burkhardt S, Benito E, Navarro Sala M, Javan SB, Haass C, Schmid B, Fischer A, Bonn S. DNA methylation changes in plasticity genes accompany the formation and maintenance of memory. Nat Neurosci. 2016 19(1):102-10
- Ivanova D, Dirks A, Fejtova A (2016) Bassoon and piccolo regulate ubiquitination and link presynaptic molecular dynamics with activity-regulated gene expression. *J Physiol* 594(19):5441-5448.
- Ivanova D, Dirks A, Montenegro-Venegas C, Schöne C, Altrock WD, Marini C, Frischknecht R, Schanze D, Zenker M, Gundelfinger ED, Fejtova A (2015) Synaptic activity controls localization and function of CtBP1 via binding to Bassoon and Piccolo. *Embo J* 34(8):1056-1077.
- 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. Journal of Visualized Experiments e51310: 2014.

- Bera S, Kreutz MR. When synaptic proteins meet the genome - Transcriptional regulation in cell death and plasticity by the synapto-nuclear messenger Jacob. Neuropsychopharmacology 39: 245-6, 2014.
- Mikhaylova M, Karpova A, Bär J, Behnisch T, Zuschratter W, Kreutz MR. Cellular distribution of the synapto-nuclear protein messenger Jacob in the rat brain. Brain Structure Function 219:843-60, 2014.
- Regus-Leidig H, Ott C, Lohner M, Atorf J, Fuchs M, Sedmak T, Kremers J, Fejtova A, Gundelfinger ED, Brandstatter JH (2013) Identification and immunocytochemical characterization of piccolino, a novel piccolo splice variant selectively expressed at sensory ribbon synapses of the eye and ear. *PLoS One* 8(8):e70373.
- Karpova A, Mikhaylova M, Bera S, Bär J, Reddy PP, Behnisch T, Rankovic V, Spilker C, Bethge P, Sahin J, Kaushik R, Zuschratter W, Kähne T, Naumann M, Gundelfinger ED, Kreutz MR. Encoding and transducing the synaptic or extrasynaptic origin of NMDA receptor signals to the nucleus. Cell 152: 1119–1133, 2013. *Research highlight in Nature Reviews Neuroscience. F1000Prime RECOMMENDED **10***, *Hot Topic Neuropsychopharmacology
- Fejtova, A.; Pothula, S.; Ivanova, D Synapse-to-nucleus signaling by CTBP1 regulates neuronal gene.expression: implications for restorative plasticity in depression. *J Neurochem, Volume: 134 Special Issue: SI Supplement: 1 Pages: 98-98 Meeting Abstract: YIC03-01 2015*
- Lazarevic V, Fienko S., Andres-Alonso MR, Anni D, Ivanova D, Montenegro-Venegas C, Gundelfinger EG, Cousin MA and Fejtova A. Physiological concentrations of amyloid beta regulate recycling of synaptic vesicles via alpha7 acetylcholine receptor and CDK5/calcineurin signaling. *Front. Mol. Neurosci.* (accepted)

Dissemination of the data

The results of the project have been presented to the scientific community in a form of oral presentations (NWG 2015, Synapse meeting Bristol, UK 2015; ISN-APSN-ANS, Cairnes, Australia, 2015 Meeting, Anatomisches Colloquium, Göttingen 5.6.2015; Summer symposium of ICN Erlangen 14.7.2017, GRK2162 retreat 13.10.2016,) and poster on many other scientific meetings.

List of press releases

- Highlighted in: Kravchick DO, Jordan BA. Presynapses go nuclear! EMBO J. 2015 Apr 15;34(8):984-6.
- 2015- Hugo-Junker Price for Research and Innovation of Country Saxony-Anhalt for discovery of the novel CtBP1 signaling pathway that links cellular metabolism and regulation of gene expression in neurons
- Leibniz Journal: Forschung aus der ersten Hand: Healthy Ageing Synaptische Aktivität reguliert den Proteinhaushalt von Neuronen
- LIN Research reports 2014/2015; Memories are made of this: Synaptic activity controls protein synthesis in brain
- LIN Press release:
  - http://www.lin-magdeburg.de/assets/files/press/2015-12-16_PI_Hugo-Junkers-Preis
  - www.lin-magdeburg.de/assets/files/press/2015-02-11_PI_CtBP1

References

Kaushik R, Grochowska KM, Butnaru I, Kreutz MR. Protein trafficking from synapse-to-nucleus in control of activity-dependent gene expression. Neuroscience 280: 340-350, 2014.

Hubler, D., Rankovic, M., Richter, K, Lazarevic, V., Altrock, W.D., Fischer, K.D., Gundelfinger, E.D., and Fejtova, A. (2012). Differential spatial expression and subcellular localization of CtBP family members in rodent brain. PLoS One 7, e39710.

Fainzilber M., Budnik V, Segal R, Kreutz MR. From Synapse to Nucleus and Back Again - Communication Over Distance Within Neurons. J. Neurosci. 31(45):166045-16048, 2011.

Jordan BA, Kreutz MR. Nucleocytoplasmic protein shuttling: The direct route in synapse-to-nucleus signaling. Trends in Neurosci. 32:392-401, 2009.

Panayotis N, Karpova A, Kreutz MR, Fainzilber M. Macromolecular Transport in Synapse to Nucleus Communication. Trends in Neurosci. 38: 108-116, 2015.

Tom Dieck, S., Altrock, W.D., Kessels, M.M., Qualmann, B., Regus, H., Brauner, D., Fejtova, A., Bracko, O., Gundelfinger, E.D., and Brandstatter, J.H. (2005). Molecular dissection of the photoreceptor ribbon synapse: physical interaction of Bassoon and RIBEYE is essential for the assembly of the ribbon complex. J Cell Biol 168, 825-836.