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Neurotranslation: An international networking initiative to target Shank-mediated neuropsychiatric disorders (Shankopathies) Antragsnummer: K204/2016

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Inhalt

1. Executive Summary 3
2. Zielerreichung und Umsetzung der Meilensteine 4
3. Aktivitäten und Hindernisse 4
4. Ergebnisse und Erfolge 4
5. Chancengleichheit 6
6. Qualitätssicherung 6
6. Zusätzliche eigene Ressourcen 7
7. Strukturen und Kooperation 7
8. Ausblick 7

1. Executive Summary

It is nowadays widely accepted that synaptic diseases, or synaptopathies, cause major psychiatric and neurological disorders. What is still missing is a frame work for translation and intervention in synaptopathies that acknowledges synapse diversity, frailty and tenacity in disease relevant circuits. The resolution of the extremely complex signaling processes, particularly with respect to neuronal circuit function and the high diversity of synapses, has remained a major obstacle. SHANK proteins are major scaffolders of excitatory synapses in the brain. SHANKs belong to the very few examples where a clear genetic linkage to synaptic dysfunction in diverse neuropsychiatric disorders, including autism spectrum disorders (ASD), schizophrenia, and Phelan-McDermid syndrome was established. Numerous pathogenic re-arrangements in the *SHANK* genes have been reported that have high clinical relevance. While gene deletions, unbalanced translocations or interstitial deletions have been studied extensively in several mouse knock out models, research on the missense mutations which result in ASD in the vast majority of cases is largely undervalued. An international team of researchers led by Dr. Michael R. Kreutz from the Leibniz Institute of Neurobiology Magdeburg (LIN), and Dr. Eunjoon Kim from the Korea Advanced Institute of Science and Technology (KAIST) tackled the question how such mutations cause autism. The mechanisms underlying these conditions, collectively termed "Shankopathies," were investigated. Molecular consequences of autism mutations in the *SHANK* genes were elucidated. To address the most pressing questions, we have used novel humanized Shankopathy mouse models for the study of neuropsychiatric disorders. In addition, mouse models with conditional alleles to study the synaptic function of Shanks in a region- and cell-type specific manner during development and adulthood were employed. Finally, major emphasis was put on synaptic dynamics of Shanks carrying mutations associated with neuropsychiatric disorders.
SHANKs are scaffold proteins that contain many binding sites for other proteins and acts as a kind of master organizer for the postsynaptic protein machinery: They link transmitter receptors, signaling molecules, and the cytoskeleton and are indispensable for the precise work of synapses. With a series of proof-of-principal study we could provide an experimental pipeline on how to link structural effects of disease-associated missense in synaptic proteins with a protein's properties in its native environment. In addition, we were able to characterize synaptic dysfunction resulting from single missense point mutation in Shank3 that causes autism in humans from the molecular to the synaptic connectivity levels. This work enabled us to decipher potential links between synaptic endophenotypes and the expression of behavioral phenotypes typical of ASD. Further work was focused on other proteins with a known role in neuropsychiatric disorders. Here we could demonstrate in many cases a causative role for synaptic dysfunction. Moreover, our studies illustrated the value of deep structural analysis of selectrd missense mutations at the level of molecular dynamics. Albeit mutations that were investigated, were located within the same domain, they had distinct effects on SHANK3 folding in vitro and kinetics at the synapse, suggesting that each new mutation needs to be studied individually and data cannot be directly extrapolated or generalized. Apart from scientists with a research focus on neuropsychiatric disorders, we anticipate interest in this work from structural biologists and researchers working on molecular dynamics simulations as

well as bioinformaticians who are looking for new relevant targets to apply artificial intelligence for protein modeling and structure predictions.

2. Zielerreichung und Umsetzung der Meilensteine

In this project we were breaking ground in the field of Shankopathies. We succeeded in the implementation and study of novel mouse models of human synaptopathies WP1 and WP2 and thereby fostered the translational research strategy of the LIN for the next coming years. The analysis of synaptic dynamics of Shanks carrying mutations associated with neuropsychiatric disorders has led to novel insights in the causes of ASD (WP3+4). Based on the implementation of novel read-outs of ASD in mice we could also establish novel interventions (WP5+6). The main milestones were the analysis of i) knockin mice harboring human point mutations that result in ASD. To develop and apply a ii) top-notch molecular dynamics workflow and biophysical analysis that allowed us to predict conformational changes in Shank3. The quantitative mass spectrometry and biochemistry data from Shank mutant mice iii) merged into a network model to understand the complexity of synaptic proteostasis in synaptopathy. Finally, iv) we analyzed synaptic dynamics of mutant Shank proteins *in vitro* and *in vivo*.

3. Aktivitäten und Hindernisse

During the course of the project we have deciphered in a team effort structural underpinnings of Shankopathies. We have developed and implemented novel technology and created novel transgenic mouse lines. In addition, we had several interactions between project partners that led to novel concepts and ideas on the cause of ASD. The project largely worked out as planned but we were facing the challenge that personnel hired for the project got promoted to the next career level. We could meet this challenge by hiring young talented researchers. We have not experienced major delays in conducting experiments in the first years of funding. From 2020 onwards, however, we had a shortage of mice and travel restrictions prevented reciprocal lab visits, workshops and personal meetings due to the COVID-19 pandemia. Even a cost-neutral prolongation of the support could only in part compensate for these problems.

4. Ergebnisse und Erfolge

SHANK3 is a multidomain synaptic scaffold protein most prominently expressed in the brain. Disruption of SHANK3 function has been linked to numerous neuropsychiatric and neurodevelopmental disorders. In fact, it is one of the few proteins with a clear genetic linkage to synaptic dysfunction in conditions like the Phelan-McDermid syndrome (PMS) and other ASDs disease states collectively coined as shankopathies. However, the data linking missense mutation-induced changes in protein structure and dynamics to the occurrence of ASD-related synaptic and behavioral phenotypes is scant. The Klm/Kreutz/Mikhaylova labs have recently shown that two ASD-associated point mutations (R12C and L68P), both located within the same domain of SHANK3 exhibit distinct changes in secondary and tertiary structure as well as higher conformational fluctuations (Bucher et al., 2021). Local and distal structural disturbances result in altered synaptic targeting and changes of protein turnover at synaptic sites (Bucher et al., 2021 / Fig. 1).

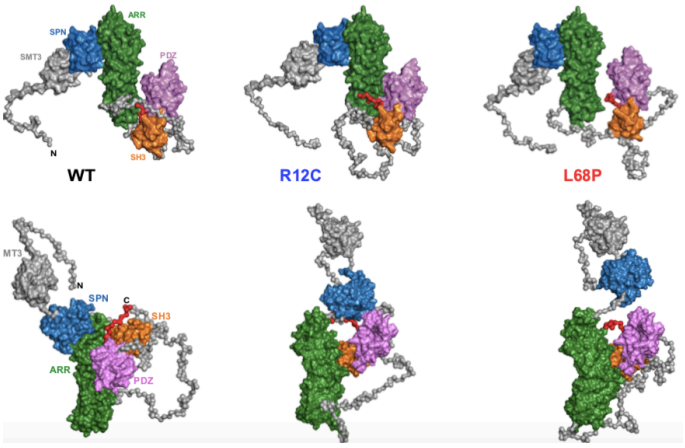

Figure 1: Rigid-body CORAL models of the SHANK3 complex topology in solution. High-resolution structures of individual SHANK3 fragments were fitted against zero-extrapolated SAXS profiles. Molecular dynamics simulations reveal subtle changes in conformation and conformational stability in SHANK3 harboring a point mutation.

In yet unpublished work we found drastically increased binding of the mutant protein to the major PSD scaffolder SynGAP. We have determined how the tremendously different binding to SynGAP that we observed alters synaptic residing time of SynGAP1 and analyzed with different means the molecular underpinnings and consequences of increased binding.

Binding studies have included surface plasmon resonance, isothermal titration calorimetry and dynamic light scattering. Biochemical assays have focused on impact for liquid-liquid phase separation and assembly of a SynGAP-Shank3 scaffold and its consequences for Ras/Rap1 signaling and the SHANK3-actin interaction, which regulates dendritic spine morphology. Finally, we have determined with STED microscopy the PSD lattice at high-resolution in spine synapses utilizing PSD-95, SynGAP, Homer and Shank antibodies. EM studies to reveal ultrastructure are currently ongoing. In addition, we have explored in SHANK3 knockin mice whether a missense human point mutation lead to changes in stability at the single-synapse level, which might result in changes of efficiency of synaptic transmission and cause ASD-related behavior. Taken together, this part of the project provided for a defined synaptopathy in human patients molecular mechanisms that bridge from synaptic endophenotypes and to behavioral phenotypes.

Further work was devoted to the role of other crucial proteins involved in synaptopathies, many of those that directly interact with Shanks (like for instance the synapto-nuclear protein messenger Jacob). A major advance was the generation of novel humanized Shankopathy mouse models in collaboration with lab of Eunjoon Kim in South Korea. Yet unpublished joined data on neuropsychiatric disorders in these animals will be submitted soon. In addition, mouse models with conditional alleles to study the synaptic function of Shanks in a region- and cell-type specific manner during development and adulthood were employed and these data will be submitted soon as well. Finally, several technological advances come to fruition now and we expect further publications to arise from 'Neurotranslation'.

Publications

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Qualifications of involved personnel

Three PhD theses have been successfully defended as part of 'Neurotranslation'. Marina Mikhaylova successfully applied to professorship at Humboldt University of Berlin.

Further dissemination

Scientific outreach at conferences -The results of the project have been presented to the scientific community in form of oral and poster presentations at many scientific meetings. Workshop - We have co-organized the 3rd International Symposium Healthy Ageing, Berlin 2019, topic “Synaptic Ageing - Challenges for Translational Neuroscience”. Meeting 'Synaptic Function and Neural Circuitry' - This meeting was co-organized with the Korean partner and took place in Busan, South Korea. The second meeting that was planned in Germany could not take place due to restrictions in the COVID-19 pandemia. In addition, several press releases dealt with the topic of 'Neurotranslation'.

5. Chancengleichheit

The Leibniz SAW consortium “Neurotranslation: An international networking initiative to target Shank-mediated neuropsychiatric disorders” implemented the general policy for equal-career management at all participating institutions. Equal opportunities were already guaranteed during the recruitment process. The consortium was additionally supported by the Office for Equal Opportunities and Career Development at the LIN, founded by the FemPower Project of the State of Saxony-Anhalt.

6. Qualitätssicherung

All consortium members committed themselves to the rules of good scientific as outlined by the DFG. Guidelines for good scientific practice have been established at the LIN and were enforced during conduct of all studies. The PhDs and postdocs supported from grant 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. 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.

7. Zusätzliche eigene Ressourcen

The consortium had technical support from three technicians. The costs of their salary amounted roughly to 100.000.- € over a period of four years. Consumables and costs for animals were covered by core funds in a similar range.

8. Strukturen und Kooperation

The project was carried out in a tight network of scientific collaborations between the partners. Core competences and technologies in the network were available for all partners. We should highlight an extensive exchange of personnel to broaden the technological competence at all institutions before the COVID19 pandemia with several personal meetings. In response to the challenges of the pandemia we had several online meetings. The consortium benefited from the supradisciplinary team, which allowed the access to high-end technology and methods. The established international network will further collaborate in the future.

9. Ausblick

The overarching conceptual framework of Neurotranslationwais rooted on the notion of 'Synaptic Frailty'. We have assumed that proteostasis is a key determinant for synaptic frailty as opposed to synaptic tenacity and that the organization and topology of the protein network in synapses impose predetermined breakpoints that are crucial for the stability of synaptic contacts. We have predicted that the molecular organization of the Shank scaffold defines tipping points for early synaptic dysfunction that will quickly escalate and prevent adaptive changes necessary for circuit function that can be best seen as defects in synaptic plasticity. An inevitable consequence is that affected synapses exhibit molecular pathology that is in functional terms visible as a loss of responsiveness to altered synaptic input and firing, i.e. impaired synaptic plasticity. In the future this will become a central point of our research and we will follow up on the hypothesis that impaired synaptic plasticity feeds back to synaptic function, disrupts synaptic neurotransmission, interrupts network function and causes early symptoms of neuronal dysfunction in ASD.