Project title:

Shedding light on plasticity of monoaminergic circuits in the brain

Project number:

J28 /2017 (SheLi)

Executive Summary

Over the past five years, our research at the Leibniz Institute for Neurobiology (LIN) has established a foundation for understanding the plasticity changes of noradrenergic circuits. In particularly we focused on changes within the context of neurodegenerative diseases. The support from the Leibniz Association and an additional 2 million euros in funding has catalyzed the establishment of a fully equipped laboratory, advancing our understanding of noradrenergic neurons and their role in Parkinson's disease. We have identified subsets of these neurons susceptible to degeneration due to high energy demands in early disease stages.
Our research has led to the development of a new functional model of the Locus Coeruleus (LC), incorporating both intrinsic cellular properties and synaptic inputs, as well as the inhibitory modulation of neighboring neurons through volume transmission. This "bystander" model has elucidated the topographic organization of the nucleus, reconciling observed synchronous activity within the LC network with limited electrical coupling between neurons.
We've successfully integrated key methodologies such as virus production, electrophysiology, fiber photometry, and molecular cloning into the portfolio of the institute. Our findings highlight the impact of altered noradrenergic signaling on cognitive flexibility and memory, shedding light on the potential early indicators of neurodegenerative diseases.
Despite facing challenges such as delays in laboratory setup due to administrative negotiations and the COVID-19 pandemic, our team has maintained productiv during this challenging time. Our progress includes significant publications, the establishment of international collaboration network, and contributions to the broader scientific community through protocol sharing and workshops.
The lab's impending closure in mid-2025 brings urgency to our ongoing projects. We aim to further elucidate the synaptic connections and neuronal circuits changes observed in the early stages of neurodegenerative diseases and continue to contribute to the field through our research outputs and international collaborations.
In conclusion, our research has not only provided insights into the functioning of noradrenergic networks but has also established a robust platform for continued scientific inquiry into the adaptive and maladaptive processes of catecholaminergic neurons, with implications for learning disabilities and early neurodegenerative diseases.

1. Achievement of objectives and milestones

The report "Shedding Light on the Plasticity of Monoaminergic Circuits," funded by the Leibniz Association, facilitated the establishment of my research program in Germany at the Leibniz Institute for Neurobiology, Magdeburg. This initial support led to the foundation of a lab and the acquisition of over 2 million euros in further funding over five years, marking a significant achievement in establishing my independent research.
Investments in virus production, electrophysiology, fiber photometry, and molecular cloning, as outlined in the SheLi proposal, have been successfully integrated into our group's workflow and the institute's operations. Our research focuses on monoaminergic circuits, especially noradrenergic neurons. A major accomplishment has been advancing the scientific community's understanding of the changes in noradrenergic circuitry and plasticity in the early stages of Parkinson's disease, linked with specific symptoms. We have shown that certain noradrenergic neuron subsets with targeted

projections are more susceptible to the diease due to high energy demands during early neurodegenerative disease stages, leading to localized cortical areas with noradrenergic axonal degeneration (Baral et al., 2021), and corresponding behavioral deficits in rodent models.
AIM I addressed controlling norepinephrine (NE) levels via chemogenetic modulation of locus coeruleus (LC) neurons projecting to the prefrontal cortex (PFC). We noted in the interim report the reduced efficiency of DREADDs over extended activation, as reported by Manvich et al., 2020. Consequently, we explored alternative chemical methods, inducing the formation of quinones from catecholaminergic metabolites via tyrosinase expression, leading to neuromelanin accumulation and cell death in noradrenergic neurons. We also adopted optogenetic strategies for precise temporal control of NE.
To assess the behavioral implications of altered NE in the PFC, we measured cognitive flexibility using a reward task requiring animals to adapt a learned strategy to a new context, a more cognitively demanding process than traditional rule switching. Animals needed multiple sessions to achieve 80% task performance, with performance depending on PFC NE levels. Using a fiber photometry system with sensors like GRAB_NE or nLight2, we found that decreased activity or loss of noradrenergic cells prolonged the learning of new strategies and adherence to outdated rules, suggesting inadequate NE levels for PFC network resetting and exploration.
AIM II investigated neural plasticity and homeostatic changes in the PFC network under varying NE levels. In collaboration with Olga Penegarakov, we introduced her behavioral phenotyping workflow into the instiute. We constructed a long-term recording arena to observe group behavior over four weeks, extracting over 30 different behaviors. This continuous monitoring linked homeostatic adjustments in the LC-NE circuitry with behavioral changes over time. Preliminary findings indicate that reduced NE affects multiple behavioral domains; notably, degenerated LC neurons disrupt sleep patterns. Structurally, we observed axonal denervation preceding cell loss, with certain neurons showing particular vulnerability, leading to a specific sequence of noradrenergic denervation across brain areas. Our goal is to determine the order of behavioral impairments to aid future clinical diagnoses.
AIM III focused on simultaneous NE release recording across multiple brain regions. This became less critical after the publication of a similar methodology and the potential recruitment of a researcher by LIN's new director, Stefan Remy. Although the researcher ultimately established their lab in Strasbourg, we are expanding our optical measurements of NE release to other brain regions like the sensory cortices and amygdala.
AIM IV initially aimed to understand the functional role of PFC efferents on GAD2 neurons in the LC's dendritic zone. However, recent publications in Science and Nature Neuroscience prompted us to redirect our focus to the role of GAD2-positive neurons in health and disease. With Prof. Glenda Halliday, we're investigating these neurons' presence in the human LC and their role in Parkinson's disease. Additionally, we discovered a new neuronal circuit motif in GAD2-positive neurons within the ventral tegmental area (VTA), which inhibits dopamine neurons and simultaneously dampens activity in uninnervated brain regions while downregulating VTA dopamine levels. This research is poised for publication and of broad scientific interest.
Financially, we adhered to the proposed budget, with two major deviations. We opted to purchase a ready-to-use fiber photometry system from Doric instead of building one, driven by the enthusiasm of a Ph.D. student to focus on research over system calibration. We also repurposed an existing two-photon system for imaging with patch-clamp capability, integrating it into our LIN setup.

2. Activities and obstacles

There were several factors leading to delays especially in the beginning of our project. For example, some of our designated rooms were entangled in negotiations, which delayed the set-up of our laboratory space. Furthermore, also during the group's initial phase, procurement regulations caused significant delays in acquiring major equipment, such as stereotaxic injectors, and notably, the aforementioned upgraded imaging system, which was postponed by a year and a half.
The COVID-19 pandemic presented another major hurdle. The LIN administration managed to keep most facilities operational and introduced staggered working hours for researchers and Ph.D.

students. Scientific personnel, including myself, were encouraged to work remotely. In hindsight, this isolation proved challenging for the entire team and led two Ph.D. students to reconsider their career paths and conclude their studies prematurely. Consequently, we had to redistribute their responsibilities to several Master's students who joined the lab.
Additionally, our lab manager, Stefanie Hillert, experienced a high-risk pregnancy and was unable to work during the final five months (from March 2019). The institute struggled to find a new lab technician due to budget allocation issues. Ultimately, we employed a technical assistant trainee, Celina Dölle, who was already within the institute. Celina performed excellently but became unavailable after a personal tragedy in September 2022. It then took another year to find a suitable replacement.
As our research is addressing forefront research questions in the field of noradrenergic modulation, a recent publication in Nature Neuroscience (Breton-Provencher et al., 2019 DIO 10.1038/s41593-018-0305-z) described a similar electrophysiological characterization of $GAD2^{+}$ neurons in the region of the peri-LC as we proposed in aim IV (proposal figure 3 B). In agreement with our unpublished results acquired during my postdoctoral work as well as preliminary measurements from HH, these inhibitory neurons and LC neurons receive synaptic input from prefrontal efferences. They are forming a feedforward inhibition circuit to precisely time the burst activity in noradrenergic neurons of the LC. Similar to our results, the article describes an increase in arousal as well as pupil dilation upon optogenetic probing of these efferences. As mention in the Zwischenbericht, that the article from Breton-Provencher missed to highlight the corresponding behavior implication of such circuit motif, and we were confident to establish these shortcomings. In 2022 Breton-Provencher published an article that specifically address the question of the behavioral correlates of the GAD2 neurons in the peri-LC.

Results and successes

As previously mentioned, our research team has contributed to a better understanding of projection-specific degeneration of locus coeruleus (LC) neurons and the associated behavioral impairments. This work has established our group's visibility within the national and international scientific communities. We have contributed to and published five research articles and one review so far.

Queiroz Zetune Villa Real K, Mougios N, Rehm R, Sograte-Idrissi S, Albert L, Rahimi AM, Maidorn M, Hentze J, Martínez-Carranza M, Hosseini H, Saal, K-A, Oleksiievets N, **Prigge M**, Tsukanov R, Stenmark P, Fornasiero EF, Opazo F. 2023. A Versatile Synaptotagmin-1 Nanobody Provides Perturbation-Free Live Synaptic Imaging And Low Linkage-Error in Super-Resolution Microscopy. Small methods. e2300218. https://doi.org/10.1002/smtd.202300218

Goldenberg AM, Schmidt S, Mitelman R, Levy DR, **Prigge M**, Katz Y, Yizhar O, Beck H, Lampl I. 2023. Localized chemogenetic silencing of inhibitory neurons: a novel mouse model of focal cortical epileptic activity. Cerebral Cortex. 33(6):2838-2856. https://doi.org/10.1093/cercor/bhac245

Baral S, Hosseini H, More K, Fabrin TMC, Braun J, **Prigge M.** 2022. Spike-Dependent Dynamic Partitioning of the Locus Coeruleus Network through Noradrenergic Volume Release in a Simulation of the Nucleus Core. Brain Sciences. 12(6):Article 728. https://doi.org/10.3390/brainsci12060728

Oppermann J, Rozenberg A, Fabrin TMC, Cabrera GC, Béjà O, **Prigge M**,
Hegemann P. 2023. Robust Optogenetic Inhibition with Red-light-sensitive Anion-conducting Channelrhodopsins. Elife. https://doi.org/10.1101/2023.06.09.544329

Wetzel N, Widmann A, Schöllkopf U, **Prigge M**, Krauel K. 2022. A new paradigm to assess pupil dilation as a marker for a dysfunctional arousal regulation in children with ADHD. PsyArXiv. https://doi.org/10.31234/osf.io/q3c9e

Damaris Holder & **Matthias Prigge**, (2022), Spatial and temporal considerations of optogenetic tools in an all-optical single-beam experiment In: Papagiakoumou E. (eds) All-optical methods to study neuronal function. Neuromethods Vol. 191, Humana Press, New York, NY.

Moreover, we have secured over 2 million euros in extramural funding from German, European, and international agencies additional to the generous starter package of the Leibniz Association. This

funding enables us to pursue our research, which concentrates on the adaptation and maladaptation of catecholaminergic neurons to external and genetic factors. We have expanded our research focus from the proposal that was centered on learning disabilities such as ADHD also to cognitive impairments that are seen in early stages of neurodegenerative diseases like Parkinson's disease.
Given our well-established laboratory infrastructure, along with the ongoing data collection and analysis, I am optimistic about the lab's increased scientific output in the future. However, since the laboratory will not remain operational at the Leibniz Institute for Neurobiology (LIN) beyond mid-2025, we are driven to complete our ongoing projects as promptly as possible.

3. Equal opportunities, career development and internationalization

Our research group has expanded to fifteen members, including Master's students, Ph.D. candidates, Postdoctoral researchers, and three technical assistants. Our team comprises ten female and five male researchers. Our inaugural Postdoctoral researcher, funded by the Alexander von Humboldt Foundation, has returned to Brazil to establish his independent research laboratory.
During the COVID-19 pandemic, two of our Ph.D. candidates decided to discontinue their current projects within our lab and join other research groups. Nevertheless, they are still pursuing academic careers.
In our lab, all researchers, starting from the Master's level, are encouraged and supported by me to contribute their unique ideas and develop their scientific projects. All of our Master's students have chosen to pursue a Ph.D. Further, they are encouraged to attend workshops and international conferences to engage in scientific discourse with their peers.
I have also initiated a biennial two-week student summer workshop focused on advanced imaging and data analysis, which can be found at www.lindoscope.com. We invite European and international students to the Leibniz Institute for Neurobiology for this workshop. It is co-funded by the European Molecular Biology Organization (EMBO) and the State of Saxony-Anhalt.

4. Structures and collaboration

LIN's research focus shifted with the inauguration of Prof. Stefan Remy as the new director in 2020. Initially, the nature of the changes was unclear, leading to uncertainty among the institute's scientific staff which created challenges for collaboration within the institute..To address this, our research group established excellent collaborations with local institutes, including DZNE (with Dr. Betts and Prof. Hämmerer) and Otto-von-Guericke University (with Prof. Braun and Prof. Dunay). Nationally, we have strong ties with Charité and Humboldt University in Berlin (with Dr. Ross, Prof. Hegemann, and Dr. Vierock). Internationally, we collaborate with renowned scientists such as Prof. Vila from Barcelona on the Neuromelanin mouse model, the Karolinska Institute for single-cell RNA sequencing, and Prof. Melitis on RNAscope analysis to study compensatory effects in noradrenergic projection areas. Additionally, we are working with Prof. Awartramani at the University of Chicago on phenotyping neuromelanin mouse models.

5. Quality assurance

To ensure the highest quality in our research, we have established a centralized cloud-based repository for standard procedures that are regularly updated and utilized by everyone in the lab to ensure reproducibility. These established protocols are also uploaded to www.protocol.io for access by the wider scientific community.
We regularly conduct progress reports where ongoing and preliminary research is openly discussed. All our publications are shared internally and are made publicly available on BioRxiv following initial journal submission. All data are stored permanently in LIN internal storing system, as well made available in a curated from via different platforms such as github, google drive or NCBI.
Animal welfare is a priority for us, but the process of obtaining an animal license is exceedingly slow, ranging from eight to eighteen months, both within our institute and at the Landesverwaltungsamt.

This delay has a significant impact on the work of Ph.D. students within the broader scope of their research. Even if the process for the animal license begins immediately after a project proposal is approved, and despite the laboratory recruitment and work having already commenced, students often cannot start their intended projects involving animal work until much later. Furthermore, additional amendments frequently exceed the legal processing period of four weeks. This leads to considerable frustration among students and postdoctoral fellows and me. Nonetheless, it is important to note that despite these prolonged approval times, the standard of animal welfare, along with the quality and reproducibility of animal experiments, is maintained at a high level

6. Additional resources

In line with the SheLi initiative, our institute has established a virus production line now utilized by various researchers at LIN. Additionally, we routinely develop new viral plasmids and optogenetic tools for both the LIN scientific community and our international collaborators. The rapid establishment of this viral production workflow in our group and the institute is partly due to the proposed lab visit at the Weizmann Institute of Science, specifically to Ofer Yizhar's lab, by our technical staff.
Moreover, we provide guidance to several labs within the institute on optogenetic and chemogenetic experiments. We also share much of the new equipment acquired through the BestMinds budget and other funding sources. For instance, we frequently share our stereotactic frames, surgical utilities, vibrotome, glass pipette puller, osmometer, various lasers for optogenetic stimulation, and our fiber photometry setup.
Over the past five years, we have secured more than 2 million in additional funding. This has enabled us to develop a highly equipped laboratory, which has positively impacted all research projects within the lab and has also had a beneficial effect on the SheLi project.

7. Outlook

Our research contributed to a better understanding on how the noradrenergic network processed brain-wide input to the noradrenergic center, and how it leads to a task-relevant and spatially-defined release of NE.
We brought forward a new functional model of the Locus coeruleus, where not only intrinsic cell properties and synaptic input dictate activity in the local network, but also an inhibition of neigbnouring neurons via volume transmission is taking into account. Our "bystander" model postulates a topographic organization of nucleus core (Baral et al. 2021). Furthermore, it harmonized recent findings where ex-vivo connectivity study of the nucleus cores reveals a limited electronic coupling between neurons (average 3 neurons), but adding our bystander model can than explain the synchronous activity of the LC seen in many studies (refs)

We also demonstrated that plastic changes within LC that can arise through early stage of degenerative disease such as Alzheimer or Parkinson disease. These changes can lead to symptoms that are idiosyncratically assigned to these desease. Our findings argue that impaired cognitive flexibility, memory impairment or attention-deficiet can also be indicative of smoldering degenerative disease. Yet, we did not achieve a clear understanding which exact synaptic connection give rise to these changes i.e. is the synaptic wiring within the LC altered or are neuronal circuits in the peri-LC area causal for this phenotype.
Our future direction will hopefully continue to address further these questions as our lab will be closing mid-2025 at the Leibniz Institute for Neurobiology.