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I am a basic, preclinical, and translational addiction researcher. My overall ambition is to understand reward processes, addictions and comorbidities on multi-systems levels and to develop diagnostic, preventive and therapeutic strategies accordingly. My strength lies in a **multi-systems level approach** with **high interdisciplinarity** and my ability to work in close coordination with physicists, chemists, mathematicians, clinicians, and the pharmaceutical industry. Hence, I do see myself as a so-called **T-researcher** with a very broad methodological approach to a research question and the ability to, when necessary, drill deep for a better understanding. I am only convinced of a scientific finding if I obtain **convergent evidence** from different research angles, different methods, and systems levels. I am even more convinced if a computational model either predicts or converges with my experimental findings.

I studied biology at the Universities of Tübingen and Munich and pursued my early training in behavioral pharmacology and neurochemistry at the Max Planck Institute (MPI) in Martinsried. Albert Herz and Toni Shippenberg became my mentors very early on and I was very fortunate to be in the right place at the right time – this was one of the three hot spots worldwide in opioid research at that time. I made a seminal discovery during my PhD, specifically the identification of the modulation of the reward pathway by the opposing actions of endogenous opioid systems. This discovery was crucial for understanding reward processes on a neurochemical level and laid the mechanistic foundation for the use of opioid antagonists such as naltrexone and nalmefene to treat relapse in alcohol-dependent patients. Both papers in which this discovery were described (one in PNAS) became citation classics with more than 2000 citations.

In 1990, I moved to the MPI of Psychiatry in Munich (the Director at that time was Florian Holsboer) and became head of the addiction research group and was awarded a lectureship in Pharmacology and Toxicology. The main focus of the institute was CRH and stress-related disorders. Although the CRH-R1 receptor was already cloned, its *in vivo* function was not well characterized. Together with Wolfgang Wurst, we developed the first CRH-R1 knockout mouse model, which confirmed the role of the receptor in stress-responses and anxiety behavior. With this work, we even scooped the research efforts of the group of Wyle Vale at the Salk Institute– who discovered CRH back in 1981 – and published our groundbreaking work in Nature Genetics. In follow-up studies, we made the very surprising discovery that the deletion of the CRH-R1 receptor resulted in enhanced stress-induced alcohol consumption, in stark contrast to the dogma that the lack or blockade of the receptor should diminish or even eliminate stress-induced effects. Despite the fact that this work was published in Science and was awarded the Sir Hans Krebs Award for its seminal gene x environment interaction discovery in rodents, the

warning sign that CRH-R1 antagonists may not be clinically useful was ignored. At that time there was no major pharmaceutical industry company that didn't have a cost-intensive CRH-R1 program in place, and all clinical studies with promising CRH candidates eventually failed. Our work in Science and other publications from our lab provided early on an explanation as to why this concept would fail, specifically that hypothalamic and extra-hypothalamic CRH-R1 receptors and receptors on different neuronal populations often exhibit diverging effects that result in a null therapeutic outcome when all receptors are pharmacologically blocked. I see this as a prime example of how preclinical research can inform human studies and that cross-talk between basic, preclinical and clinical researchers is required to properly interpret results from biobehavioral studies. Thus, I relocated to the Central Institute of Mental Health in Mannheim in 2000 to do translational research in coordination with clinical psychologists and clinicians.

At the CIMH, I developed two major research lines –the role of glutamatergic plasticity in addictive behaviors and the interaction of clock genes and psychiatric disorders. It was more than surprising to learn that clock genes not only control our day/night rhythm, but are also critically involved in stress- and drug-related disorders. Our main findings were published in PNAS and Nature Medicine, received several awards, supported the clinical utility of agomelatine, and provided major input to the field of chronopharmacology.

Our research efforts on characterizing glutamatergic plasticity with new conditional mouse models confirmed the critical role of NMDA receptors on dopaminergic neurons in drug-seeking behavior (work published in Neuron and Nature Neuroscience) and laid the foundation for the clinical testing of NMDA receptor antagonists such as neramexane that possess fast on/off kinetics on the receptor. Although the initial clinical trial failed, our follow-up work demonstrated that this was simply a dosing failure, and that these drugs may be especially useful as substitution agents for heavily alcohol-dependent patients.

Our team research efforts also contributed to the clinical development of other anti-relapse medications, and a series of our studies were essential for providing the preclinical information for the EMA approval of acamprosate (calcium-(N-acetylhomotaurinate). However, the mode of action remained obscure until 2006, when we demonstrated that acamprosate acts on a hyper-glutamatergic status in the addicted brain. In collaboration with several clinicians, this finding was then confirmed with glutamate spectroscopy in patients. Later on, we made the very surprising discovery that N-acetylhomotaurinate is a biologically inactive molecule. Thus, the effects of acamprosate in 1.8 million treated patients can be attributed to calcium – a finding that was then confirmed with clinicians in several human experimental studies. This finding has wide-reaching implications as the calcium content of tap water can have an impact on alcohol consumption on a local population level, as well as demonstrating that the galenic formulation of generic drugs can influence the effectiveness of a given medication. This is a very much overlooked problem, especially in Germany, where cheap generic drugs that differ from the original galenic formulation often lead to complaints of non-effectiveness by patients that are often ignored. Again, this work was awarded with several scientific prices. In summary, in medication development I and my team have made exceptional translational contributions, also demonstrated by the fact that we have developed DSM-based animal models to study addictive behavior and other psychiatric disorders. For example, our animal model for alcohol addiction has become the gold standard for the pharmaceutical industry. In collaboration with several pharmaceutical companies, we have tested over 50 different putative anti-relapse compounds in this model, some of which were, or are being, clinically further developed.

It is self-explanatory that not all this research could have happened without great team members, major collaborative networks, and excellent grant support. For many years, my research team was front-runner in grant support at the CIMH. My team coordinated EU, ERANET, BMBF and DFG grants, and I am especially grateful for receiving the DFG Reinhardt Koselleck Award for innovation in neuroscience. This grant award was given for a longitudinal translational neuroimaging approach for studying disease trajectories in DSM-based animal models. This study has already and will for years continue to provide major insights into the anatomical, functional, and molecular trajectories of addictive behavior.

Key output of the years 2020-now:

1. **The IoP is moving from 3R to 6R:** Scientists working with laboratory animals are obliged to follow the so-called "3R principle": **r**efinement, **r**eduction and replacement. For many years, our animal experiments at the IoP have been committed to the 3R principles. This is best exemplified by current statistics demonstrating that in recent years we successfully reduced the number of animals used for basic and preclinical research by 47%. Our 3R activities at the CIMH were honored by the European FISEA Award and the DFG "Ursula M. Händel Animal Welfare Prize" to Hamid Noori.

   For the future, there is still further 3R potential to unlock. Due to the close proximity of basic and preclinical research sites and high interdisciplinarity in the Rhine-Neckar Region, I developed the idea to join forces and establish a common 3R Center between the CIMH and the two medical and the biosciences faculties of the Heidelberg University – referred to as the **3R Center of the Rhine-Neckar Region**. Since the beginning of 2021 our 3R Center has been funded by the MWK (Ministry of Research) of Baden-Württemberg for a 5-year period (with 0.5 million Euro) and is now coordinated by Marcus Meinhardt from the IoP (https://en.3r-rn.de). Our center activities are already well-connected to other 3R centers and platforms across Europe (for further information see the narrative report from Marcus Meinhardt).

   We have now extended our 3R approach to 6R, to include **r**obustness, **r**egistration and **r**eporting, all of which aim to safeguard and increase the scientific value and reproducibility of animal research. For this new concept, I recently published a perspective article in which I summarized the "*Ten Points to Improve Reproducibility and Translation of Animal Research*". Personally, I put a great deal of effort into writing this perspective (published in Front Behav. Neurosci. 2022) and I see it as a must-read for everyone doing animal research.

2. **Implementing the EQIPD (ENHANCING QUALITY IN PRECLINICAL DATA) Quality System at the IoP:** To facilitate the daily application of the 6R principles, we have also established the EQIPD quality system (https://elifesciences.org/articles/63294#content) to improve the documentation and reproducibility of our research. To that end, we hired a quality manager in 2022 – Björn Gerlach –a founding member of EQIPD now responsible for making the research data at the IoP traceable, transparent and reproducible at the highest academic quality level. To achieve this, Björn Gerlach now provides training opportunities for all coworkers of the IoP. We have also integrated eLabFTW, which is a free and open source electronic lab notebook, for all our projects and research activities. Our goal with EQIPD is to

provide a **blueprint for the highest quality standards** and **reproducibility in preclinical research for the CIMH** and other academic institutions.

3. **Multi-side phase II preclinical studies – a new module in the drug development process:** One new module to **further enhance the translational value of our preclinical studies** is the implementation of preclinical multi-center randomized controlled trials (preclinical RCTs) in rats as preclinical confirmation of findings. The preclinical trial design of these studies follows the guidelines established for the development of medicinal products for the treatment of alcohol addiction provided by the European Medicines Agency (EMA) and adhere to the standards proposed for confirmatory biomedical research for nonclinical laboratory studies. Furthermore, the publication of results will strictly follow the ARRIVE guidelines. We have now established a **national/European multi-side team** that involves the Charité (Christine Winter), University of Erlangen (Christian Müller), INSERM UMRS 1247 in Amiens (France, Mickael Naassila), and the University of Camerino (Italy, Roberto Ciccocioppo). Large cohorts of rats (up to n=240 male and female rats) are usually tested in a preclinical RCT design at 3 or more sites. We aim to confirm the hypotheses that treatment with **novel synthetic oxytocin receptor agonists, psychedelic drugs (such as psilocybin and R-ketamine), and exosomes** will have long-lasting positive effects on relapse behavior in our animal model of alcohol addiction. For these efforts we have received several grants in the past 3 years (e.g., ERANET PsiAlc (www.psialc.org), BMBF: Target-OXY for testing oxytocin receptor ligands, and AhEAD for testing exosomes – all projects coordinated by the IoP).

   We also focus on generalizability of the findings derived from our preclinical RCTs. By using the **unique outbred N/NIH heterogeneous (HS) rat panel** we can draw general assumptions for translation to the very heterogeneous human condition of alcohol addiction. HS rats are derived from 8 inbred rat strains and are the most highly recombinant rat intercross available (www.ratgenes.org). Together with the NIDA center for GWAS (Robert Palmer), we are performing whole genome sequencing for each tested rat and can thus perform GWASs for alcohol relapse and respective pharmacoresponses. The advantage of such a preclinical GWAS is that all environmental variables are well-controlled. From this major collaborative effort, one key publication is on its way and we strongly believe that with this new module will improve the drug development process and **produce more valuable and translatable information for clinical testing**. Furthermore, with individual genetic information available, our approach will yield tailored pharmacological interventions.

4. **Towards a better understanding of the mode of action of psychedelic drugs in alcohol addiction:** With our ERANET PsiAlc consortium we have now made major contributions to the use of psilocybin and other psychedelics (including R- vs. S-ketamine) in alcohol addiction. In a diseased brain state, psilocybin not only acts through 5-HT2_A but also via mGluR2, and our findings published in Science Advances (2022) show that mGluR2 is a molecular target for treating reduced cognitive flexibility, craving, and relapse responses. Furthermore, we demonstrate the utility of a **FDG-PET biomarker strategy to identify mGluR2** (i.e., also psilocybin) -responsive individuals. However, our most recent translational neuroimaging study in alcohol-dependent rats (currently under review at Science Advances) suggests that a standard clinical dose of psilocybin may not be sufficient to treat severe AUD cases. Therefore, **higher doses**

of psilocybin may be required for the successful treatment of AUD, which should be considered for future human trials.

5. **Building a unique in *silico* databank for genetic, epigenetic, and transcriptomic signatures for AUD and different SUDs:** In the past 3 years we have established a world-wide collaborative network to obtain human biomaterial from post-mortem brain tissue from deceased AUD/SUD patients (in collaboration with Marcella Rietschel). In our brain bank, we have now material from **483 case/controls with 1750 different brain tissue samples**. Using bulk tissue analyses, we have already successfully retrieved common and distinct epigenetic and transcriptomic signatures of AUD, tobacco- and cocaine-use disorders. These signatures are currently backed up by omics analyses in brain organoids (collaboration with Philipp Koch) and in our DSM-based animal models for alcohol and cocaine addiction. However, one limitation of bulk tissue analysis is the heterogeneity of cell types. Therefore, we have now also moved towards **single cell/nuclei multiome analysis** of our valuable biomaterials.

6. **The role of the oxytocin system in addiction and chronic pain:** We have characterized with Valery Grinevich a **new synthetic oxytocin receptor agonist** with exceptionally strong effects in our animal models of addiction. The oxytocin field has suffered from a lack of selective and BBB-penetrable ligands – with these new ligands that we have tested a new era of oxytocin research in different brain diseases will open up. With respect to the contribution of the Heidelberg collaborative center on chronic pain – which we are part of for 7 years – we have also demonstrated the role of the oxytocin system in the paraventricular thalamus in mediating the effects of childhood adversity on the development of a **chronic pain** condition.

Final statements: There is much more to report but space limitation does not allow it. Our work on **childhood adversity has demonstrated life-long changes on emotional, motivational and social behaviors, as well as on the development of a chronic pain condition** - work performed in the context of our graduate school GRK2350 (https://www.grk2350.de) and our collaborative research center on chronic pain (http://www.sfb1158.de). Another major contribution is our efforts in conducting **systematic reviews (SRs) and meta-analyses (MAs) on basic, preclinical and clinical data**. Although these are well-established tools for clinical studies, only in recent years has interest in performing SRs and MAs developed in the basic and preclinical worlds. We were front-runners in this respect, as we published our first MA on cocaine-induced dopamine release (measured by microdialysis) back in 2008. Since then we have performed more than 30 SRs and MAs. Very recently, we have also started to perform analyses of human clinical trial data and have now published **the most comprehensive MA on medical cannabinoids** in which we performed **a pharmacology-based SR and MA for all relevant medical indications**. We were very close to publishing this masterpiece in Lancet but were rejected during the final editorial stage; now it is published in BMC Medicine (2022; Bilbao and Spanagel). Also of importance is that I have scientifically advised **EMCCDA** and **WHO** with respect to new psychoactive drugs – work that I now continue for the German **BfArM**. Germany is in the **legalization process of cannabis** and I am grateful to be one of the experts that advises the government in this respect. The most important milestones for the next months is the successful application of an **ERC Advanced Grant on the role of neuronal ensembles in addictive behavior** and the **TRR 265** (https://sfb-trr265.charite.de/en/). I will be the designated spokesperson for the next 4 years for TRR 265, and it is an enormous challenge to harmonize

research plans and activities of 51 PIs in Berlin, Dresden, and CIMH to be awarded a successful second funding period on the topic of "Losing and Regaining Control in Addiction".