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Monitoring complex behavioral profiles of mouse models of psychiatric diseases: a systems-biological approach

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Table of Contents

Abbreviations

bHLH	Basic Helix-Loop-Helix
CDA	Canonical Discriminant Analysis
CNS	Central Nervous System
Cry1/2	Cryptochrome 1/2 (gene)
CRY1/2	Cryptochrome 1/2 (protein)
GWAS	Genome-Wide Association Study
PCA	Principal Component Analysis
PsyCoP	Platform for Systematic Semi-Automated Behavioral and Cognitive Profiling
RDoC	Research Domain Criteria
Tcf4	Transcription Factor 4 (gene)
TCF4	Transcription Factor 4 (protein)

List of Publications

Publication 1 (as referred to in this thesis):
Volkmann, P., Stephan, M., Krackow, S., Jensen, N., & Rossner, M. J. (2021). PsyCoP – A Platform for Systematic Semi-Automated Behavioral and Cognitive Profiling Reveals Gene and Environment Dependent Impairments of Tcf4 Transgenic Mice Subjected to Social Defeat. Frontiers in Behavioral Neuroscience, 14, 267. https://doi.org/10.3389/fnbeh.2020.618180

Publication 2 (as referred to in this thesis):
Hühne, A., Volkmann, P., Stephan, M., Rossner, M., & Landgraf, D. (2020). An in-depth neurobehavioral characterization shows anxiety-like traits, impaired habituation behavior, and restlessness in male Cryptochrome -deficient mice. Genes, Brain and Behavior. https://doi.org/10.1111/gbb.12661

Publication 3 (see appendix):
Stephan, M., Volkmann, P., & Rossner, M. J. (2019). Assessing behavior and cognition in rodents, nonhuman primates, and humans: Where are the limits of translation? Dialogues in Clinical Neuroscience, 21(3), 249–259. https://doi.org/10.31887/DCNS.2019.21.3/mrossner

1. Introduction

1.1 Tackling Psychiatric Disorders

Psychiatric disorders are a major global challenge for healthcare systems of the present and the future. Depending on interpretation of published data, it is estimated that mental illnesses are responsible for more than 14% of overall years lived with disability (GBD 2019 Mental Disorders Collaborators, 2022). This burden is likely to even increase within the next years and decades. One crucial factor in understanding disorders and developing novel treatment strategies is filling the gap between molecular insights and clinical observations (Smoller et al., 2019). Animal models are a well-established tool that promises to lead us to a more profound understanding of underlying mechanisms potentially correlating cellular and network modelling with findings in patients (Jones et al., 2011; Milton, 2022), and further guiding us towards new treatment regimens (Howland et al., 2019; Nestler & Hyman, 2010).

One way of addressing the translational gap between animals and humans is modelling specific aetiologies and pathologies of disorders in animals. For example, one general hypothesis is that two hits are necessary to lead to the onset of a psychiatric disease like schizophrenia (Bayer et al., 1999; Khan & Powell, 2018; Maynard et al., 2001).

1.2 Investigating Schizophrenia

Schizophrenia is a psychiatric disorder with three pathognomonic symptom domains, including positive symptoms like hallucinations, negative symptoms like reduced affect and lack of motivation, and cognitive symptoms, such as attentional or memory deficits (Tamminga & Holcomb, 2005). In humans, it affects both sexes equally and has two typical peaks of onset in women (adolescence and age of 30), whereas in men this peak only lies in the adolescence. The disorder occurs cross-cultural with a lifetime prevalence of approximately 1% (Kahn et al., 2015). About two thirds of patients are affected by relapses or even chronical progress with serious restrictions. Stigmatization and exclusion are possible and severe social consequences (Belvederi Murri & Amore, 2019; van Os & Kapur, 2009).

Schizophrenia is a heterogenous disease accompanied by a variety of symptoms and characterized by several subforms. As it has a clear genetic contribution, but concordance rates in monozygotic twins are considerably lower than 100% (Hilker et al., 2018), it most likely cannot be explained by

one single pathological cause, but rather time critical hits affecting and disrupting the individual's neurobiological development. This might even mean, that the current definition of schizophrenia comprises a variety of clinically similar diseases with slightly different origins (Zoghbi & Lieberman, 2018). Dissecting putative mechanisms of the diseases' development could help us distinguish these overlapping entities and identify novel treatment targets.

1.3 Two-Hit Hypothesis of Schizophrenia / Introduction of *Tcf4*

As elaborated above, it is assumed that schizophrenia might be caused by two hits that only lead to the onset of the disease when occurring conjointly (Khan & Powell, 2018).

The first hit is the genetic disposition of an individual, well characterized through several genome-wide association studies (GWAS). In my first publication, we present results of mice transgenic for Transcription Factor 4 (*Tcf4*), a well-known and recent schizophrenia GWAS hit (Pardiñas et al., 2018).

TCF4 (capitalized to indicate the protein) is a basic helix-loop-helix (bHLH) transcription factor involved in developmental and plasticity-related transcriptional programs in the CNS (Quednow et al., 2014). Besides other functions, it mediates diverse cell proliferation associated processes, regulates pathways relevant for cognition (Li et al., 2019), and acts as a transcriptional hub for several bHLH proteins. The gene coding for TCF4 lies on chromosome 18 for both mice and humans. Haploinsufficiency in the latter leads to severe intellectual disability and retardation (Pitt-Hopkins-Syndrome) (Goodspeed et al., 2018). *Tcf4* knock-out mice suffer from severe brain defects. Mild under- or overexpression, however, induces rather schizophrenia like endophenotypes (Quednow et al., 2014). As we conceived our study, a fully comprehensive phenotypic characterization had been lacking so far.

The second hit is defined by the environmental conditions individuals are exposed to, i.e., any detrimental circumstances an individual might be confronted with especially during developmental stages (Howes et al., 2017; Wahbeh & Avramopoulos, 2021). In my first publication, we made use of the so-called Resident-Intruder-paradigm to model psychosocial stress during the neurobiologically vulnerable phase of adolescence by intermittently exposing animals to social defeat (Hammels et al., 2015).

Neither one of the two hits nor the simple addition, it is hypothesized, may be sufficient to induce disruption of brain developmental processes sufficient for causing a disease. It is rather the

interaction of the two hits and its consequences that lead to the onset of psychiatric disorders (Khan & Powell, 2018; Tost & Meyer-Lindenberg, 2012; Wahbeh & Avramopoulos, 2021).

1.4 Emerging Tools for Dissecting Pathologies

Still, there is lack of comparability and need for standardization of execution, analysis, and interpretation of behavioral testing in mouse lines which in return should account for the complexity of psychiatric diseases, e.g., by considering aforementioned paradigms.

To fully grasp all aspects of an animal's phenotype, it is an established procedure to arrange behavioral tests in an arrayed pipeline (Hölter et al., 2015). The order of such pipelines typically accounts for severity of tests on animals and has the advantage of reducing animal numbers since all animals run through the whole battery of tests. By increasing redundancy of testing, it is possible to overcome limitations of weak individual paradigms. Inclusion of (semi-)automated monitoring systems, such as the IntelliCage (Kiryk et al., 2020), further improves reproducibility not only between mouse lines, but also between laboratories. These aspects facilitate designing a heterogenous and diverse testing battery generating complex datasets. Further advantages and shortcomings of such a testing battery have been unfolded in greater detail in a publication that I co-authored (see appendix) (Stephan et al., 2019).

The physiology based RDoC concept (Insel et al., 2010) then offers a valuable framework to classify assessed experimental measures through implementation of distinct functional behavioral systems. It defines the following domains: cognition, social processes, sensorimotor system, positive and negative valence, and arousal/regulation. These domains enable the description of phenotypes and parallel routine diagnostic criteria from the clinic. Hence, translation of observations in animals to disorders described in humans is facilitated (Anderzhanova et al., 2017).

Subsequently but also in general, psychiatric mouse models ask for multimodal interpretation of data which has to be informed by according statistical analysis (Tanas et al., 2022). To examine the accuracy of our and prospective other hypotheses, complex analyses of different data dimensions - amongst family-wise comparisons - are necessary to identify clusters or patterns of phenotypic entities. These types of analyses can reveal and identify an array of information necessary for deeply understanding behavior and emergent features of neurobiological physiology and pathology.

Since these demands have been addressed insufficiently so far, my project and the publications presented strived for conceiving a comprehensive testing battery suited for animal models of

psychiatric diseases, overcoming several disadvantages of testing animals in single experiments only, and embedding obtained results in a solid statistical and conceptual framework. Simultaneously, we wanted to identify a behavioral endophenotype of *Tcf4* transgenic mice exposed to psychosocial stress that might be relevant for understanding psychiatric disorders and schizophrenia in particular. Our experimental design aimed at dissecting gene x environment interactions and adequately investigating corresponding phenotype relationships.

1.5 Validation of PsyCoP by Investigating a Mouse Model with Circadian Deficiency

To further validate the construction of our phenotyping pipeline and to gain more insight into a mouse model of another relevant psychiatric symptom complex, we tested a mouse model of deficient circadian rhythmicity, namely mice deficient of the central clock genes Cryptochrome 1 and 2 (*Cry1/2*), in parallel. Thereby and in contrast to the investigation of *Tcf4* mice, we were able to elucidate the potential contribution of a disruption in a well characterized physiological brain mechanism, the circadian clock, to a particular set of psychiatric symptoms without any prior disease-specific assumptions.

The circadian clock as a means to adapt an organism's behavior to environmental changes (Asher & Schibler, 2011; Reppert & Weaver, 2002) heavily influences activity and behavior of each individual cell in the body. Through its daily oscillations it also governs brain activity and behavioral processes (Partch et al., 2014), while the suprachiasmatic nucleus functions as the central pacemaker in the mammalian system (Welsh et al., 2010). Disturbance of this system has been implicated in a variety of different clinically relevant contexts (Boivin et al., 2022; LeGates et al., 2014; Walker et al., 2020). In this study, we aimed at generating a comprehensive characterization of the behavior in *Cry1/2* deficient mice and dissecting its psychiatric phenotype which had been lacking thus far. Briefly, CRY1/2 (capitalized to indicate the proteins) are part of the transcriptional-translational feedback loop driving the circadian clock (Reppert & Weaver, 2002). Its genetic deactivation stops the entire feedback loop that is driving the circadian pacemaker. As *Cry1/2* deficient mice pose an entirely different entity of possible pathologies leading to psychiatric disease, investigating this mouse model marks a fundamentally different approach in dissecting pathogenesis in Psychiatry and thus proofed to be valuable to test construct validity of PsyCoP. Making use of the testing battery conceived within the PsyCoP framework, we were able to reveal

an extensive phenotypic profile of this relevant mouse model in chronobiology and confirm the usefulness of our experimental approach in different pathologies.

1.6 Scope of the Publications

In my first-authored publication (Volkmann et al., 2021), we introduced our platform for systematic semi-automated behavioral and cognitive profiling, in short: PsyCoP, that allows for examination of an abundance of mouse models and a better understanding of the individual phenotype as well as the relationship of and differences between various disease models. PsyCoP presents a diverse panel of well-established behavioral tests assessing different domains and their traits. It offers mapping of extensive phenotypes in mouse models of interest and subsequently facilitates displaying the results individually, within certain subdomains (e.g., endophenotypes), or as an array offering a synopsis of an animal model's overall behavioral habits. Investigation of animal models within PsyCoP may be hypothesis-driven, even excluding tests or analysis features, and aiming for revelation of expected or well-known characteristics; however, it may also be exploratory looking for patterns or striving for an overview of a novel animal model that may create new hypotheses in the first place. PsyCoP comes with an analysis pipeline ranging from structured data generation to primary processing and filing, simple visualizations, statistical analyses (family-wise group comparisons) and finally more advanced, complex, and multi-dimensional tools (heatmap, PCA, CDA) unfolding the full potential of an in-depth behavioral characterization. PsyCoP not only accounts for genetic mouse models, but also considers environmental factors in the broadest sense (as described above) or treatment strategies, thus accounting for the complex aetiology and treatment regimens of psychiatric disease. It provides a framework for setting up a comprehensive behavioral profiling and later allows for reliable comparisons of manifold different models.

We examined mice transgenic for *Tcf4* exposed to psychosocial stress in order to assess the validity of our testing pipeline as well as to investigate whether our mouse model might serve as a valuable disease model for understanding an endophenotype of schizophrenia. Our results show that cognitive deficits in flexibility learning as well as fear memory in *Tcf4* gain of function mice, as shown previously in (Brzózka & Rossner, 2013), were even enhanced for animals exposed to psychosocial stress. Therefore, this mouse model offers great potential for future research, as cognitive symptoms in schizophrenia patients lack effective treatment so far (Goff et al., 2011;

Martínez et al., 2021). A recent study already made use of the established mouse model and PsyCoP, showing that a therapeutical intervention could alleviate cognitive symptoms in stressed *Tcf4* mice (Stephan et al., 2022).

Our mice also showed *Tcf4* gene dosage dependent deficits in sensorimotor gating, again validating previous results (Brzózka et al., 2010), whereas positive and negative valence systems were solely affected by social defeat. Even more importantly, PsyCoP proved to be a valuable tool for phenotyping a mouse model with reasonable face and construct validity, as tests assessing similar traits through different paradigms pointed into the same direction.

In my second publication (Hühne et al., 2020), we examined mice deficient of two of the core circadian clock proteins, CRY1/2, therefore unable to exhibit internal circadian rhythmicity. We saw an emergent lack of rhythmicity in the behavior of our mice regarding their day-night activity patterns. In the cognitive and social domain, we only found mild abnormalities, namely in tests assessing working memory and social interaction. Strikingly, *Cry1/2* deficient mice exhibited a remarkable anxiety driven behavior with consistency across tests which could not be assigned to deficits in locomotion. Moreover, we observed a consistent restlessness within different behavioral paradigms. To identify a molecular surrogate for the observed behavior, we were able to determine higher c-Fos expression levels (an indirect marker for neuronal activity) (Cosi et al., 2021; Greenberg & Ziff, 1984) in the amygdala upon exposure to an anxiogenic stressor compared to wildtype animals. The provided study underlined the relevance of circadian disturbances in the pathogenesis of psychiatric symptoms and diseases, moreover, suggesting that patients with anxiety might profit from chronotherapy in certain settings.

Although published slightly later than the second publication of this thesis, (Volkmann et al., 2021) sets the stage for a multitude of follow-up studies. By introducing a comprehensive battery of behavioral assays and a standardized analysis and data visualization pipeline, it provides the necessary means to screen for relevant mouse models of psychiatric diseases, enabling researchers to identify target endophenotypes for potential rescue and/or treatment studies. (Hühne et al., 2020), on the other hand, takes advantage of the provided testing battery and investigates a thus far insufficiently characterized mouse model of circadian deficiency, further capitalizing on the value of such a comprehensive and standardized approach.

2. My Contribution

For the first publication (Volkmann et al., 2021), Moritz Rossner conceived PsyCoP in the first place. Niels Jensen contributed conceptually and supported me with planning of breeding of *Tcf4* mice. With guidance of Moritz Rossner and Niels Jensen, I re-established the social defeat paradigm in the lab. I conducted all experiments, taught at the beginning, and later partially assisted by Marius Stephan. Furthermore, I created large parts of the graphics in Fig.1 as well as the first heatmap and PCA analysis, which was later modified by Marius Stephan. Marius Stephan pre-processed raw data and conducted family-wise group comparisons as well as the second heatmap analysis and CDA. I drafted the first version of the manuscript and participated in improving and refining texts, tables, visualizations, and general conception equally to Marius Stephan and Moritz Rossner. Sven Krackow provided statistical expertise, helped adjusting analysis tools, and contributed to the manuscript. As Marius Stephan and I both carried out to the experimental work and data analysis - taken together to the same extent - we share the first authorship.

For the second, co-authored publication (Hühne et al., 2020), Dominic Landgraf conceived the study. The experimental design was based on prior work from (Volkmann et al., 2021), with major input from Moritz Rossner regarding the testing battery. Together with Anisja Hühne, I conducted all the behavioral experiments and animal preparation for molecular experiments, which itself were carried out by Anisja Hühne. Marius Stephan assisted with and contributed to data analysis. I created the graphics of Fig. 6B. All authors contributed to writing of the manuscript.

3. Summary (English) / Abstract

The investigation of aetiology and treatment of psychiatric symptoms and diseases remains difficult. Animal models still provide a useful tool of investigation with high translational value, bridging the gap from molecular insights to clinical observations. Nonetheless, major challenges persisting are improvements of validity of investigated animal models accounting for the complexity of pathogenesis and increasing comparability of obtained results in the face of a current lack of standardization of experiments and analysis.

With the publications presented in this thesis, my colleagues and I introduced a novel testing battery called PsyCoP, assessing behavior and cognition in mouse models of psychiatric diseases. Not only do our models account for genetic disposition, but also environmental conditions (two-

hit mouse model), as in the first publication we exposed mice transgenic for *Tcf4*, a schizophrenia risk gene, to psychosocial stress in the neurodevelopmentally vulnerable period of adolescence. Our mouse models do not aim for modelling the entirety of a disease's symptom spectrum, but rather model certain endophenotypes that may be seen as a feature of a given disorder or the representation of a patient subgroup. PsyCoP is able to identify endophenotypes through consideration of a systems-biological framework. The analysis pipeline provided – consisting of R scripts executing group comparisons, heatmap visualizations and dimension reduction techniques (PCA and CDA) – further helps standardizing interpretation of the generated data and facilitates follow-up studies, e.g., treatment experiments, by making results more comparable and comprehensible. Furthermore, workload for future studies is reduced through identification of disease-relevant conditions and corresponding abnormalities in defined domains. In our first PsyCoP paper, we were able to identify such a disease model in stressed *Tcf4* mice (namely, cognitive deficits dependent on the interaction of *Tcf4* and stress) and simultaneously prove the validity of our pipeline and the paradigms utilized. In the second paper, we profited from the conceptual work of PsyCoP's testing battery and investigated a mouse model with deficiency of the circadian clock, namely the core genes *Cry1/2*. This study proofed to be the first one providing a fully comprehensive phenotype of the *Cry1/2* mouse model. We were able to reveal a disease phenotype that might offer an interesting explanatory framework and a treatment target for certain psychiatric patients suffering from anxiety and restlessness.

4. Summary (German) / Zusammenfassung

Grundlagenforschung zu Ätiologie und Behandlung psychiatrischer Symptome und Erkrankungen stellt sich nach wie vor als herausfordernd dar. Tiermodelle sind dabei weiterhin von großem Wert und besitzen das translationale Potential, die Lücke zwischen Erkenntnissen aus der molekularbiologischen Forschung und klinischen Beobachtungen zu schließen. Die größte Herausforderung besteht aktuell darin, die Validität der untersuchten Tiermodelle zu verbessern, um der komplexen Pathogenese psychiatrischer Erkrankungen Rechnung zu tragen, und die Vergleichbarkeit von publizierten Ergebnissen zu erhöhen, indem Experimente und Analysen standardisiert werden.

In den in dieser Arbeit vorgestellten Publikationen haben meine Kollegen und ich eine neu konzipierte Testbatterie namens PsyCoP vorgestellt, die Verhalten und Kognition in Mausmodellen psychiatrischer Erkrankungen untersucht. Unser Tiermodell berücksichtigt dabei nicht nur genetische Prädisposition, sondern auch Umweltbedingungen im Sinne eines two-hit Mausmodells. In der ersten Publikation setzten wir für ein Schizophrenie-Risikogen transgene Mäuse (*Tcf4*) in der vulnerablen Phase der Adoleszenz psychosozialem Stress aus. Unser Mausmodell hat dabei nicht den Anspruch, das gesamte Symptomspektrum einer Krankheit wie Schizophrenie abzubilden, sondern es erfasst vielmehr einen umschriebenen Endophänotypen, der entweder der Untersuchung von definierten Symptomkomplexen einer bestimmten Erkrankung oder von Untergruppen von Patientenkollektiven dienen kann. Mit PsyCoP können wir ebendiese Endophänotypen im Rahmen eines systembiologischen Ansatzes identifizieren. Die entwickelte Analysepipeline - bestehend aus R-Skripten, die Gruppenvergleiche, Heatmap-Visualisierungen und Dimensionsreduktionstechniken (PCA und CDA) umfassen - hilft darüber hinaus, die Interpretation der generierten Daten zu standardisieren und erleichtert Folgestudien, z. B. Behandlungsexperimente, indem Ergebnisse leichter vergleichbar und nachvollziehbar werden. Darüber hinaus wird der Arbeitsaufwand für zukünftige Studien durch die Identifizierung krankheitsrelevanter Domänen in umschriebenen Bereichen reduziert. In unserer ersten PsyCoP-Publikation konnten wir ein klinisch relevantes Krankheitsmodell in Stress-exponierten *Tcf4*-Mäusen identifizieren (i.e. kognitive Defizite nur durch die Interaktion von *Tcf4* und Stress) und gleichzeitig die Validität unserer Pipeline und der verwendeten Verhaltens-Paradigmen nachweisen. In der zweiten Publikation profitierten wir von der konzeptionellen Vorarbeit an der PsyCoP-Testbatterie und untersuchten ein Mausmodell mit defizienter zirkadianer Uhr durch Fehlen der Gene *Cry1/2* (essenzieller Teil der molekularen zirkadianen Uhr). Diese Studie ist die erste ihrer Art, die eine umfassende phänotypische Charakterisierung des *Cry1/2*-Mausmodells liefert. Wir konnten einen pathologischen Phänotyp aufdecken, der ein interessantes Erklärungsmodell und Behandlungsziel für bestimmte psychiatrische Patienten mit Angst und Unruhe darstellen könnte.

5. Publication 1

Volkmann, P., Stephan, M., Krackow, S., Jensen, N., & Rossner, M. J. (2021). PsyCoP – A Platform for Systematic Semi-Automated Behavioral and Cognitive Profiling Reveals Gene and Environment Dependent Impairments of Tcf4 Transgenic Mice Subjected to Social Defeat. Frontiers in Behavioral Neuroscience, 14, 267. https://doi.org/10.3389/fnbeh.2020.618180

6. Publication 2

Hühne, A., Volkmann, P., Stephan, M., Rossner, M., & Landgraf, D. (2020). An in-depth neurobehavioral characterization shows anxiety-like traits, impaired habituation behavior, and restlessness in male Cryptochrome -deficient mice. Genes, Brain and Behavior. https://doi.org/10.1111/gbb.12661

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9. Appendix

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