CANDY

Comorbid Analysis of Neurodevelopmental Disorders and epilepsY

Deliverable D1.4
Report on the critical developmental windows for treatment intervention

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1 Executive summary

To determine the development trajectory of co-morbidity phenotype expression on the developmental trajectory in animal models, RUG performed assessments of sensory processing and behavioral phenotypes in the Shank3 and Nrxn1 genetic mouse models at various development stages: 6-8 weeks, 12-14 weeks, and >18 weeks. In addition, electrophysiological (EEG) assessments focusing on sensory processing have also been performed in the Nrxn1 genetic mouse model at four time points: 6-8 weeks, 10-12 weeks, 14-16 weeks, and 18-20 weeks of age. These studies have revealed an interesting developmental sensory profile that shows slightly different trajectories across models. Based on the developmental sensory trajectories and brain analyses we have implemented an intervention study to target these sensory phenotypes via a synaptic protein at the 12 weeks' time point as a function of the Shank3 genotype. The proof of concept intervention study revealed that somatosensory targeting of the cofilin synaptic protein pathway did not change the sensory phenotype in this genetic mouse model.

2 Introduction

Genome-wide association and sequencing studies of people with autism spectrum disorder (ASD) have implicated various genes to the diagnosis. In workpackage (WP) 1, we studied specific risk genes in isolation to try and understand some of the mechanisms underlying comorbid phenotypes. In particular, the following genes have been studied:

1. Shank3: is a major scaffolding protein located in the postsynaptic density and essential for synaptic functioning. Deletions in SHANK3 can result in Phelan McDermid syndrome which is often accompanied by ASD as well as resulting in other symptoms such as intellectual disability or language and speech difficulties.
2. Nrxn1: is a large presynaptic protein. Found to be affected not only in ASD, but also in schizophrenia. The gene has been associated with changes in synapse formation and synaptic transmission, thus mutations may affect the communication between neurons.

The overall aim of this study was to first determine if and when during development certain changes in phenotypes might arise in these knockout models and if these changes are followed or preceded by biochemical changes during these developmental stages. Based on this data, our next aim was to provide a proof-of-concept intervention study by targeting the brain and behavioral phenotypes observed during different developmental time points in genetic mouse models.

As part of WP1, task 1.3 aimed at establishing whether developmental critical periods are relevant for the efficacy of interventions in the models developed in Task 1.1 (also see Deliverable 1.1). Indeed, brain development is initially determined by distinct temporal and spatial stages of gene expression and intrinsic neuronal activity and targeting these phases during the development of neuronal circuits likely underpinning neurodevelopmental disorders (NDDs) could influence the efficacy of pharmacological interventions.

Please note that, considering the Dissemination level of this report (Public Deliverable), and that the results of the work hasn't been published yet and will be included in an upcoming paper, Partner RUG, lead of the deliverable, has decided not to present the full data set. More results are available in the technical reports."

3 Main section

3.1 Readouts and developmental windows for intervention study

Two types of readouts are discussed below in order to establish whether developmental critical periods are relevant for the efficacy of interventions in the models developed in task 1.1 of CANDY: 1- the study of the level of proteins that contribute to synaptic integrity in different brain regions of *Shank3* and *Nrxn1* mice models by means of Western Blot, and 2- neurological morphology analyses.

3.1.1 "Protein synthesis" readout in Shank3 and Nrxn1 mice models

We have studied brain and blood biomarkers from the *Nrxn1* and *Shank3* genetic models at two developmental stages. This was performed in parallel to behavioral assessments. Brain markers were studied in brain areas relevant for sensory symptoms as well as other ASD-related behavioural differences, namely in the prefrontal cortex, somatosensory cortex, thalamus and striatum. By means of western blotting, protein levels in these brain areas were determined, specifically of proteins important for synaptic plasticity. For each genetic line (*Nrxn1* and *Shank3*) we measured brain biomarkers in WT and KO mice at two developmental time points, 6 and 12 weeks, representing early adolescence and early adulthood respectively. This analysis has revealed specific differences in synaptic brain markers only at the 12-week time point specifically in the *Shank3* model. Therefore, together with the behaviour data in these models, the 12-week time point was selected as the sensitive developmental window to initiate an intervention study by targeting the synaptic protein that was altered as a function of the Shank3 genotype. An intervention study has now been completed during which synaptic integrity is targeted at 12 weeks in the *Shank3* model. Wild type and Shank3 homozygous gene knockout animals received a local viral vector mediated intervention in the somatosensory cortex (either with a cofilin construct or a sham construct (see for methodological details also Riemersma et al., Cerebral Cortex, 2024). This study in Shank3 gene knockout mice revealed that somatosensory interventions with a synaptic protein (i.e., cofilin) did not alter the sensory phenotype in the Shank3 mouse model.

3.1.2 "Neurological morphology" readouts

Considering that our intervention did not alter the Shank3 sensory phenotype, no subsequent morphological analyses have been performed.

4 Conclusions

The goal of this work package is to determine the critical developmental windows for treatment intervention. First, developmental trajectories of animals with genetic mutations related to ASD were determined. The two models described here are the Shank3 and Nrxn1 genetic knockout mice. Changes in behaviour were characterised for both gene models. This was supported by biochemical

analysis of brain tissue. Additionally, electrophysiology revealed a developing sensory phenotype for the Nrxn1 model. These electrophysiological findings were published recently (Østergaard and Kas, Frontiers in Neuroscience, 2025). Together, this resulted in a sensitive time window that marked the time point for an intervention study targeting a synaptic protein that was altered at 12 weeks of age as a function of the Shank3 genotype. The proof of concept intervention study revealed that somatosensory targeting of the cofilin synaptic protein pathway did not change the sensory phenotype in this genetic mouse model.

5 Glossary

Abbreviation / acronym	Description
ADHD	Attention Deficit/Hyperactivity Disorder
ASD	Autism Spectrum Disorder
ID	Intellectual Disabilities
NDDs	Neurodevelopmental Disorders
Nrxn1	Neurexin 1
KO	Knock-out
SHANK3	SH3 And Multiple Ankyrin Repeat Domains 3
WP	Work-Package
WT	Wild-type