CANDY

Comorbid Analysis of Neurodevelopmental Disorders and epilepsY

Deliverable D1.1
Report on developmental trajectories in NRNX1 and SHANK3 genetic mouse models

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1 Executive summary

To determine the developmental trajectory of co-morbidity phenotype expression and the modulatory role of neuroinflammation on the developmental trajectory in animal models (tasks 1.1 and 1.2.) we performed assessments of sensory processing and behavioural 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. Brain tissue and blood samples for assessment of neuroinflammatory factors was analysed in 6- and 12-week-old animals. Based on the developmental sensory trajectories and brain analyses we will be testing time-dependent drug efficacy (tasks 1.3 and 1.4) on these behavioural and electrophysiological phenotypes by targeting pathogenic processes.

2 Introduction

Genome-wide association and sequencing studies of people with autism spectrum disorder (ASD) have implicated various genes to the diagnosis. The phenotype is most likely caused by a complex cocktail of all these genes.

In workpackage 1, we studied specific genes in isolation (namely Shank3 and Nrxn1) to study developmental trajectories of sensory phenotypes.

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. Please note, that in WP1 male mice are studied as ASD is considered more common in males. However, studies in WP2 are investigating both males and females. With this comparison, the CANDY project is able to investigate, analyse and impute sex biased differences in the animal models.

Nrxn1: is a large presynaptic protein. Found to be affected not only in ASD, but also in schizophrenia. The studies are correlational studies, showing that people with a diagnosis have mutations in Nrxn1. The gene has been associated with changes in synapse formation and synaptic transmission, thus mutations may affect the communication between neurons.

The aim of this study was to determine if and when during development certain changes in sensory phenotypes might arise in these knockout models and if these changes are followed or preceded by biochemical changes during these developmental stages.

Our hypothesis is that sensory phenotypes can be observed throughout development in different sensory modalities in our knockout mice and that changes in biochemical pathways precede these phenotypes.

3 Methods and Results

3.1 Behaviour

The sensory trajectories in *Shank3* and *Nrxn1* genetic knockout mice have been assessed, including the assessment of responsiveness to tactile and auditory stimuli. More specifically, the sensory assessment consisted of a von Frey test in which fibre filaments are applied to the hindpaws of the mice to assess tactile sensory sensitivity. To assess auditory sensitivity, we tested the acoustic startle response to single stimuli ranging from 65dB (background) to 120dB. In addition, we performed a prepulse inhibition test in which single as well as paired stimuli were presented to the mice to test inhibition of the startle response when a prepulse is present. Prepulse consisted of a 74-86dB stimulus and the startle response of a 120dB stimulus. A multi-sensory prepulse inhibition task was also performed in which the air puff consisted of a tactile stimulus (air puff) combined with a 120dB startle stimulus. These behavioural sensory phenotypes have been assessed at various developmental stages, ranging from early adolescence to late adulthood (6, 12 and 18 weeks). These studies have revealed an interesting developmental sensory profile that shows slightly different trajectories across models. Differences in both tactile and acoustic sensitivity have been observed in the knockout mice. These early sensory phenotypes progress over time and become more pronounced as a function of aging.

3.2 Biochemical readouts

In parallel to the behavioural assessment, we have also studied brain and blood biomarkers from these genetic models during these developmental stages. This has revealed differences in brain markers across models at specific developmental time points (i.e., specific changes in cofilin at week 12 of development in the Shank3 model). Together, this will help identify targets and critical periods for intervention.

Faeces samples have also been collected and the analysis is pending (WP3).

3.3 Electrophysiology

For the *Nrxn1* genetic knockout mice a study was carried out examining the neuronal response to visual and auditory stimuli. The responses were recorded via a chronic implant with electrodes over the visual and auditory cortices, while the animals were awake and freely moving. The recordings were carried out at four time points when the animals were 10, 14, 18 and 22 weeks of age. For the auditory stimulation a 10 kHz tone was played for 50 ms, either repeated every 3 s or with different intervals varying from 100 ms to 1 s, to test the integrity of low-level sensory processing respectively sensory filtering of stimuli. These paradigms were matched in the visual domain by a 10 ms flash of light presented every 1 s or at intervals of 100 ms to 500 ms. The repetition of the stimuli allows for averaging the response to remove noise from the signal.

The results show that the male Nrxn1 homozygous knock-out animals become more excitable over the course of development. (In a single case, this hyperexcitability led to two absence seizures being detected). Additionally, the phenotypes varied over sensory modality, in general, the phenotype

developed over the course of development. It is important to realize that most of the electrophysiological genotype effects were seen after the originally planned two time points. For that reason, we expanded the electrophysiological recordings in the Nrxn1 mice for two additional time points later in life (weeks 18 and 22). Addition of these later time point assessments revealed an important observation, namely that these sensory phenotypes develop at relatively late developmental stages. To compensate for this additional electrophysiological work in the *Nrxn1* model, we decided to investigate and report in *Shank3* mice only the behavioural assessments of the developmental sensory profiles. Both electrophysiological assessments in Nrxn1 and behavioural assessments in *Nrxn1* and *Shank3* models point to a progressive development of sensory phenotypes for a variety of sensory modalities.

4 Conclusions

The goal of this work package is to characterise developmental trajectories of animals with genetic mutations related to ASD. The two models studied here are the *Shank3* and *Nrxn1* genetic knockout mice. Developmental sensory phenotypes were observed based on both behavioural and electrophysiological assessments. Based on these analyses and additional biochemical analyses in brain tissue from these mouse lines, a vector mediated intervention in the *Shank3* model is planned for Q1 and Q2 of 2023.

5 Glossary

Abbreviation / acronym	Description
ASD	Autism spectrum disorder
Nrxn1	Neurexin 1
KO	Knock-out
SHANK3	SH3 And Multiple Ankyrin Repeat Domains 3