

Marie Curie International Reintegration Grant: Final report

Investigating the role of Neuroligin-4 in the development of autism-related synaptic and behavioral abnormalities in mice

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Autism is a prevalent neurodevelopmental disorder that is currently estimated to affect 1 in 110 children. The biological basis for autism remains largely unknown, and no cure or effective pharmacological treatment is currently available. It is therefore essential to expand our understanding of the molecular mechanisms underlying autism spectrum disorders (ASDs) in order to identify novel therapeutic targets that can be used for the development of treatment strategies. A significant number of mutations linked to autism affect proteins that are essential in the development or function of synapses, and abnormalities in synaptic structure and plasticity are observed in numerous animal models of autism and related disorders. Together with the fact that the onset of autism occurs during a key period of synaptogenesis in the human brain, these findings indicate that autism may be a 'synaptopathy', a disorder resulting from the abnormal development and function of synapses. Accordingly, one important approach to understanding the biological basis of autism is to investigate the mechanisms by which the molecules linked to autism affect synaptic development and function. One of these molecules is Neuroligin 4 (NL4), a member of the Neuroligin family of synaptic cell adhesion proteins. At least 15 different independent mutations and copy number variations in the NLGN4 locus have been linked to autism, making loss-of-function mutations in NL4 one of the most frequent monogenic causes of non-syndromic autism known to date. However, very little is currently known about the role that NL4 plays in synapse development and function, or how loss-of-function mutations of NL4 may affect synapses to cause the cognitive and behavioral impairments associated with autism. In this project, we aimed to investigate this problem using an approach based on NL4 knockout (KO) mouse models.

The first objective of this study was to identify which synapses in the forebrain express NL4, and to characterize the synaptic effects of NL4 deletion in mice. NL4 was found to be widely expressed throughout the forebrain at low levels, but particularly prominently in specific sub-regions of hippocampus, somatosensory cortex and globus pallidus. Focusing further on the hippocampus, a brain region known for its role in learning and memory, we found that NL4 loss specifically impairs the composition and function of perisomatic inhibitory synapses, which are involved in modulating and fine-tuning the processing of cognitive information. These synaptic impairments were accompanied by substantial changes in the oscillatory network properties of the hippocampus, which are known to be essential for a number of cognitive functions such as the integration of sensory, spatial and temporal information to guide behavioral performance. These findings provide an important basis for understanding the molecular mechanisms by which NL4 mutations may modify cognitive functions, and for the development of potential pharmacological treatments that specifically target these inhibitory synapses.

In parallel, our research has opened several new avenues for future studies on Neuroligins and autism. We have conducted a proteomics screen in NL4 KO mice that has generated an interesting collection of candidate proteins with altered expression in the absence of NL4, and

we will continue to pursue these observations in the future. We have also developed novel approaches to the study of social interactions in mice, which will both expand our own research on the NL4 autism model and benefit other researchers involved in studying autism-related behavioral phenotypes in mouse models.

Our second objective was to develop new research tools to study the temporal requirement for Neuroligins in synapse development and function. Specifically, we were interested in the following two questions: (1) Are Neuroligins required during early postnatal development for the initial formation of synapses, during later development and adulthood for the maintenance of synapses, or both? (2) Can synaptic and behavioral phenotypes that arise due to early loss of Neuroligin function be rescued later in postnatal development? This latter issue is particularly important with regard to treatment strategies for autism, since it will be very important to know whether and which impairments can be reversed later in life. In order to begin to address these questions, we generated new mouse models in which Neuroligins can be conditionally deleted or activated after prior shut-down in order to achieve specific expression of Neuroligins at different time points in postnatal brain development. These mice will provide valuable tools for future studies both for our own projects and for other researchers interested in the role of Neuroligins in synapse development and neurodevelopmental disorders.

Overall, this research has significantly advanced our knowledge on the role of NL4 at synapses relevant to autism-related behaviors. It has also provided the fellow with an excellent basis upon which to continue this line of research, both in terms of future experimental plans and with respect to her integration into the European autism research community. The funding provided by the Marie Curie International Reintegration Grant has therefore substantially promoted the fellow's long-term career perspectives to establish an independent research program on the role of synaptic dysfunction in cognitive and social impairments in mouse models of autism.