2022 Best Research Poster Award

Barwon Health

DEAKIN UNIVERSITY

An exciting new tool for neuroscience: reverse engineering viral induced synaptogenesis

Stephanie Keating[1,2*], Katie Ardipradja[1], James Broadbent[3], Utpal Bose[3], John Bingham[1], Vinod Sunaramoorthy[1,2]

1. Diagnostics Surveillance & Response, CSIRO Australian Animal Health Laboratory, East Geelong, VIC, Australia
2. School of Medicine, Deakin University Waurn Ponds, VIC, Australia
3. CSIRO Agriculture and Food, University of Queensland, St Lucia, QLD, Australia

INTRODUCTION

Synapses are the junctions between neurons that permit the transmission of electrical signals enabling our body to function.

Lack of synapse development or degeneration can lead to neurological disorders which can have a detrimental impact on a patient's life.

We have recently discovered that the glycoprotein from a highly neuroinvasive strain (HNS) of rabies virus has a natural ability to induce the formation of new synapses (synaptogenesis) and filopodia to enable efficient transfer of virus between neurons.

OBJECTIVES/AIMS

1. Investigate the region/domain within the HNS glycoprotein responsible for synapse formation
2. Investigate the host neuronal proteins influenced by the glycoprotein to increase synapses.

We aim to use this natural ability of HNS rabies glycoprotein to understand synapse formation and develop potential therapeutics to enhance synaptic development

METHODS/RESULTS

To study the glycoproteins derived from the low neuroinvasive strain (LNS) and HNS strains, lentivirus carrying the glycoprotein transgene tagged with GFP from each strain were generated and primary embryonic mouse neurons were infected. The cells were then imaged through live cell confocal microscopy.

SCAN QR FOR LIVE CELL IMAGING VIDEOS

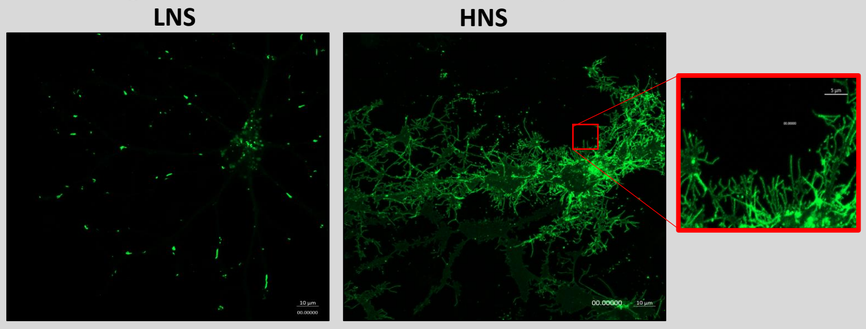

Figure 1. Live cell imaging of primary embryonic mouse neurons transfected with low neuroinvasive strain (LNS) and HNS rabies glycoproteins.

The neurons expressing HNS glycoprotein produced many dynamic filopodia seeking synaptic contacts with neighbouring neurons. The neurons were then fixed and stained with a synaptic antibody and imaged through confocal microscopy. The HNS transfected neurons had a significantly higher count of synapses in comparison to the LNS.

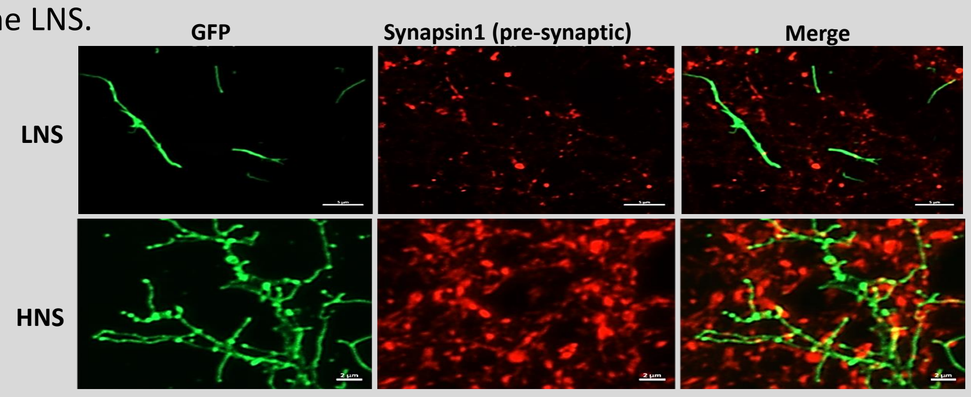

Figure 2. Confocal imaging of primary embryonic mouse neurons transfected with LNS and HNS glycoproteins.

A genetic analysis was then performed on the LNS and HNS glycoproteins to identify mutations responsible for the increase in filopodia and synaptogenesis. 19 mutations were identified and integrated into the LNS strain to determine which mutation/s were responsible for the HNS phenotype. Confocal microscopy then showed that only one mutation caused an increase in filopodia and synaptogenesis.

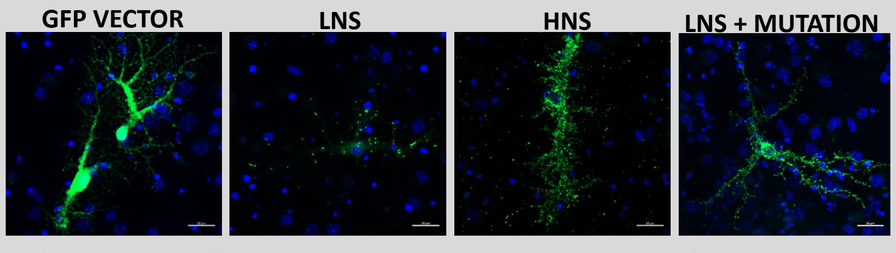

Figure 3. Confocal imaging of primary embryonic mouse neurons transfected with the LNS glycoprotein integrating the 19 mutations.

METHODS/RESULTS

Proximity labelling was then performed in neurons expressing the LNS and HNS rabies glycoprotein in order to identify proteins interacting with glycoprotein to induce synapse and filopodia formation

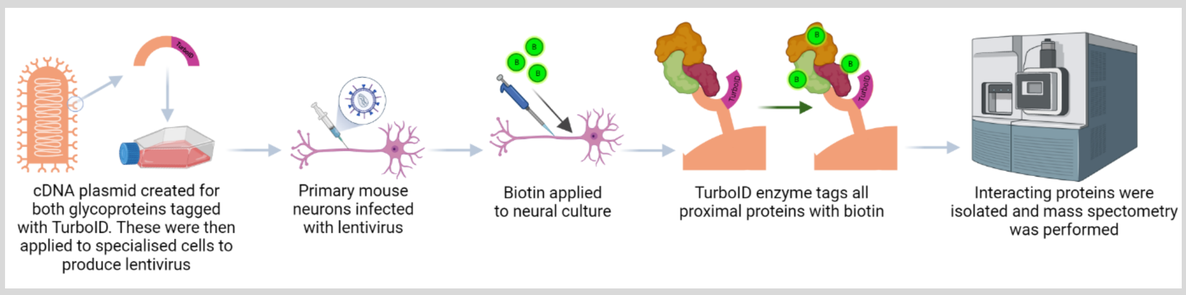

Figure 4. Diagram outlining transfection and proximity labelling protocol

Several proteins were identified in the HNS mass spectrometry results that are responsible for synapse formation and stabilization.

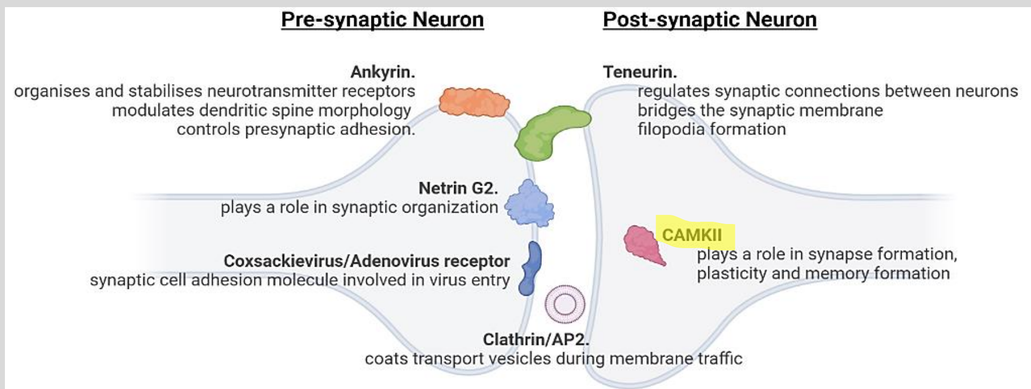

Figure 5. Localisation of synaptic proteins identified in HNS mass spectrometry

CAMKII is an important protein that plays a key role in the formation and plasticity of synapses. We hypothesised that HNS glycoprotein influenced CAMKII, enabling the overproduction of new synapses in comparison to LNS infected neurons. We therefore treated HNS infected neurons with a CAMKII inhibitor drug KN-93 before imaging through confocal microscopy.

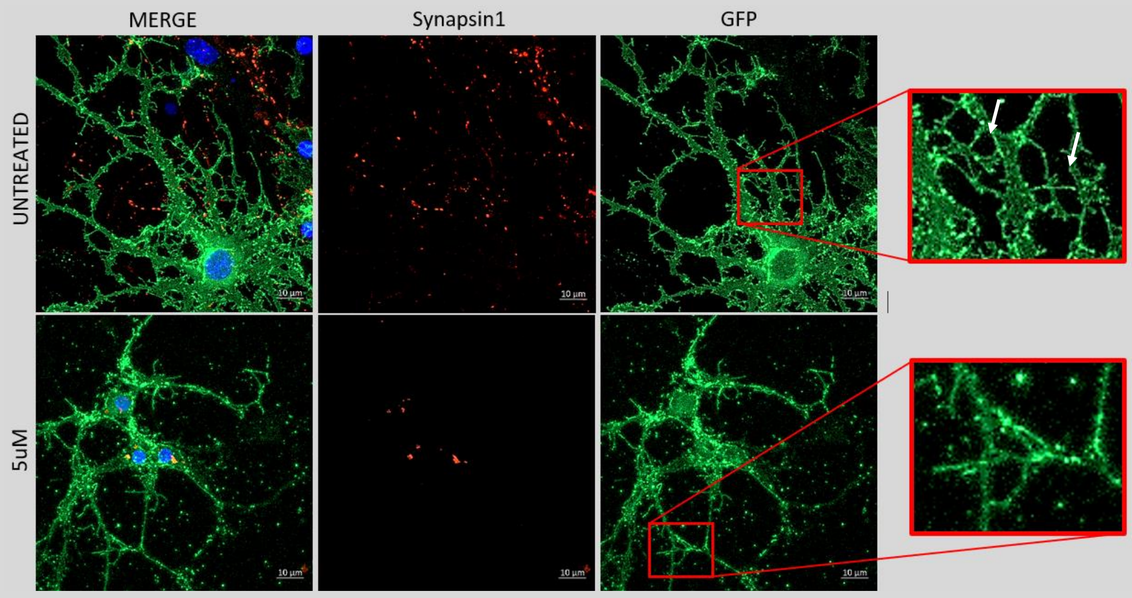

Figure 6. Confocal imaging of neurons transfected with HNS and treated with CAMKII inhibitor

At 5uM, we saw the loss of filopodia and a drastic reduction in synapses. This allowed us to conclude that the ability of HNS rabies glycoprotein to increase synapse formation is dependent on CAMKII activation.

DISCUSSION/CONCLUSION

We are now expressing smaller peptide derivatives encompassing the filopodia and synaptogenesis inducing mutation in neurons. These experiments will help us to design next generation therapeutics to increase synapse formation and improve synapse functioning in neurological disorders.

We also plan to perform western blott and confocal analyses to determine other synaptic proteins that are drastically upregulated in the HNS strain. This will help us to gain novel insight into new signaling pathways and molecules that are involved in synaptogenesis and rabies transmission.

REFERENCES & ACKNOWLEDGEMENTS

I would like to acknowledge the ACDP histology team and CSIRO facility staff for their support and expertise.

I would also like to thank the small animal facility at ACDP for providing our mouse embryos and assisting us with our ethics approval.

DEAKIN UNIVERSITY

CSIRO