Optimisation of viral transduction in rodent cortical cells

Sophie Graham*, Carolina Gandara, Gavin Clowry

MBBS Medicine 170058744 s.graham5@newcastle.ac.uk

Introduction

Epilepsy is a neurological disorder characterised by recurrent seizures due to bursts of increased electrical activity in the brain. Focal epilepsy describes those originating from one specific area. Several trials of new therapies are aiming to target only this seizure focus, to increase efficacy and minimise potential side effects.

One such therapy employs a technique called **optogenetics**[1]. A virus engineered to contain genes to make cells responsive to light is injected into the seizure focus, and specifically infects neuronal cells. Transduction involves the transfer of these genes from the virus into the nucleus of the target neurons, resulting in production of proteins called opsins on the cell membrane. If successful, shining light using a small electrode inserted into this area may alter neuronal activity and inhibit a seizure.

Optogenetics is a relatively new method, currently being studied primarily in rodent models of epilepsy. Though previous trials have been successful[2], work is ongoing to improve the technique. Our goal was to investigate the potential to increase levels of transduction by using a range of concentrations of three viral vectors, aiming to maximise opsin production while also identifying any negative impact caused by viral infection.

Aims

1. Is there an optimum type or concentration of viral vector for infection?
2. What extent of toxicity, if any, is caused by infection?

Methods

1. Cells from the cerebral cortex of rats at embryonic day 19 were cultured in vitro for 6 days. These were divided into three cultures and each infected with a different virus: AAV1 and AA8 – both forms of adeno-associated virus – and a lentivirus, all containing genes encoding different opsins. Cultures were all infected at the same range of viral concentrations diluted in cell growth media.
2. Cell cultures were fixed and stained using immunocytochemistry, which attaches fluorescent tags to specific proteins and allows visualisation with a microscope. Antibodies were used to tag NeuN (a specific neuronal cell marker), Hoechst (binds to the DNA of all cell types), and the viruses.
3. Remaining neurons in both infected and non-infected cell cultures were quantified to assess any cell loss as a result of infection.

Results

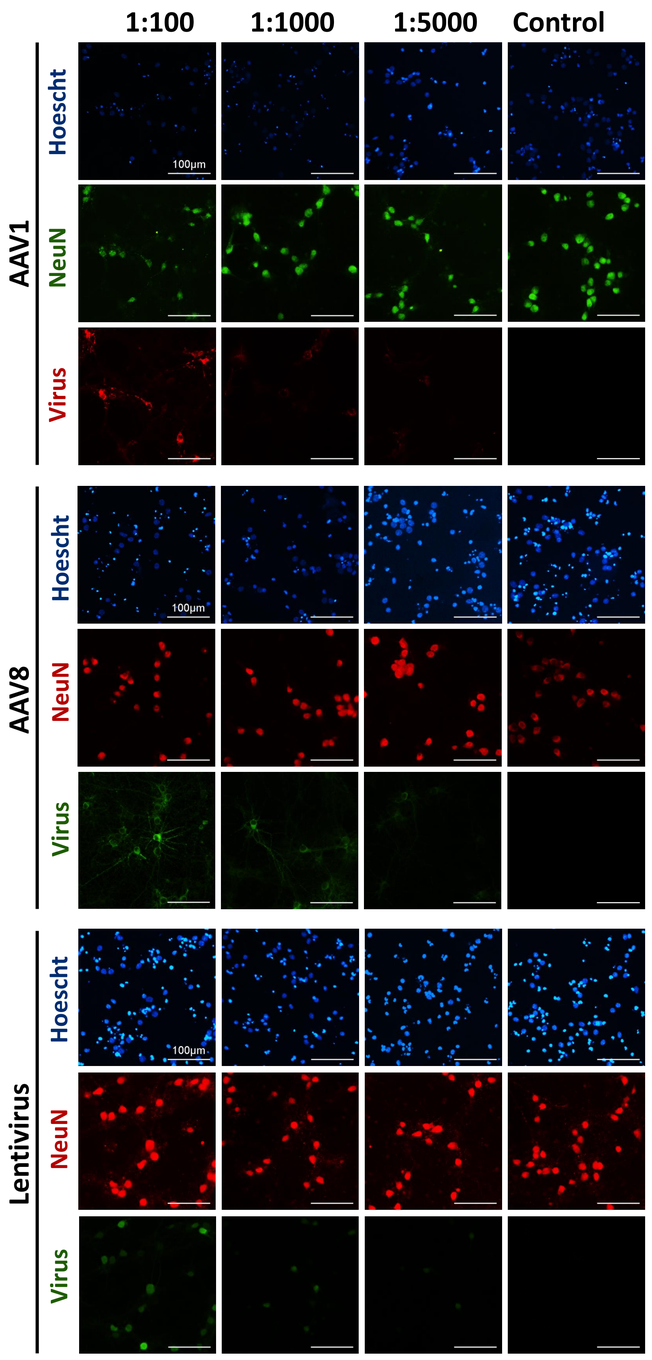

Figure 1: Immunofluorescent staining of cell cultures from highest concentration to no infection (control). Anti-mCherry (red) and anti-GFP (green) antibodies used as fluorescent tags for NeuN and viral-infected cells. All scale bars 100µm.

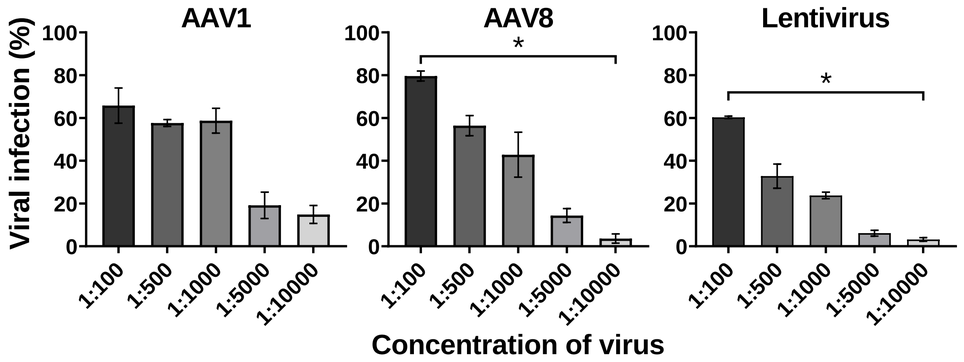

Figure 2: Quantification of viral infection of cell cultures at decreasing concentrations.
Error bars represent standard error of mean (SEM). * represents statistical significance ($p < 0.05$).

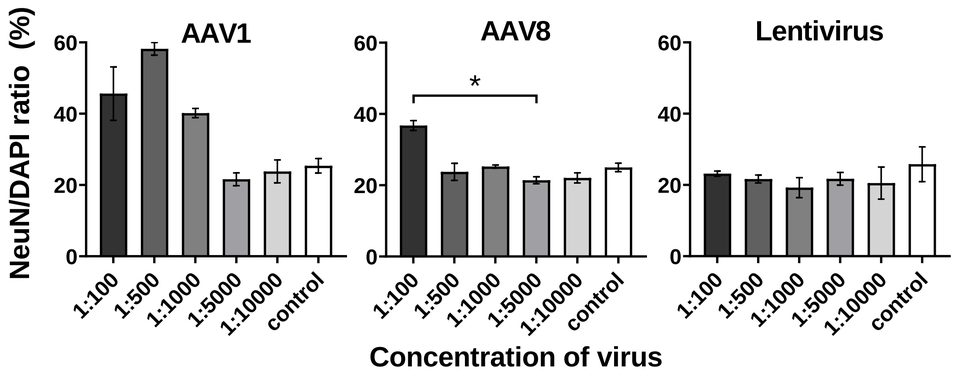

Figure 3: Quantification of neuronal cells relative to total cells following viral infection.
Error bars represent standard error of mean (SEM). * represents statistical significance ($p < 0.05$).

Discussion

- Viral transduction was most successful in cultures infected at 1:100, particularly AAV8 (Figs. 1 and 2), though this was significant only with AAV8 and Lentivirus ($p < 0.05$).
- A general decline in neuronal cells relative to the total number of all cells was observed particularly with AAV1 and also AAV8 (Fig. 3), though overall there was little significant difference between cultures, suggesting any toxicity from infection was minor.
- Results from this work are optimistic, though highlight that the relationship between rate of transduction and effect on cultures is not necessarily consistent and warrants further study.

Acknowledgements

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References

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2. Francis TC, Chaudhury D, Lobo MK. Optogenetics: illuminating the neural bases of rodent behavior. *Open Access Anim. Physiol*. 2014;6, 33-51.