Long-term expression of transgene in targeted cortical neurons following inoculation in the mouse striatum of a non-replicative HSV-1

J Ratelade[1] | J Basset[1] | T Jégu[1] | S Salot[1] | A Epstein[1]

1: EG 427 - 29 rue du faubourg Saint Jacques – 75014 Paris, France
Corresponding author: Julien Ratelade – julien@eg427.com

Introduction

Efficient targeting of cortical neurons in central nervous system (CNS) remains a significant challenge for gene therapies. Herpes simplex virus type 1 (HSV-1) is a human neurotropic virus that establishes productive infections in epithelial cells and latent infections in neurons. Non replicative (nr) HSV-1 based vectors have been developed to exploit their therapeutic potential by leveraging their neurotropism, safety, and long-term transgene expression abilities. In contrast to AAVs, nrHSV-1 vectors can efficiently transduce neurons of the CNS at very low doses and can be efficiently transported retrogradely to connected brain regions. Here we administrated a nrHSV-1 vector in the striatum to broadly target, by retrograde transport, cortical neurons projecting in this area.

Methods

We constructed a nrHSV-1 vector named EG143A with deletions in the essential ICP4 and ICP27 genes and several other non-essential genes (UL55, UL56, as well as one copy of ICP34.5, ICP0 and LAT). This vector expresses the mGreenLantern reporter gene under the control of the non selective CAG promoter. The transgene is located in the LAT (Latency associated transcript) region (Figure 1). This vector was injected in the striatum of mice (4E+06 PFU in 2 µl). Biodistribution pattern and kinetics of mGreen lantern was analyzed by fluorescence imaging and digital droplet PCR (ddPCR) (Figure 2).

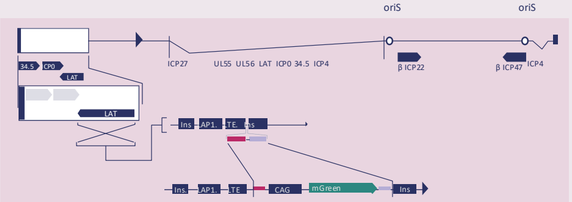

Figure 1 | Genomic map of EG143A vector

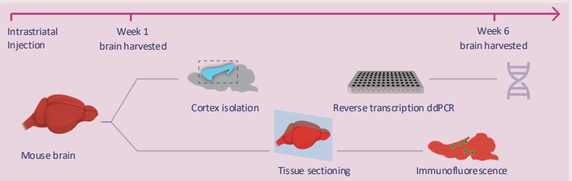

Figure 2 | Experimental design

1 Intrastriatal administration of nrHSV-1 vector leads to efficient transduction of cortical neurons by retrograde transport

EG143A was injected in the striatum of mice. After one week, mGreen lantern was expressed in striatum, substantia nigra, and cortex suggesting that the nrHSV-1 vector transduces neurons at site of administration and projecting neurons by retrograde transport. In the cortex, EG143A demonstrated high-level transgene expression in layer V cortical neurons. Transgene expression was seen in many cortical areas such as the anterior cingulate, orbital, somatomotor, somatosensory and entorhinal cortex.

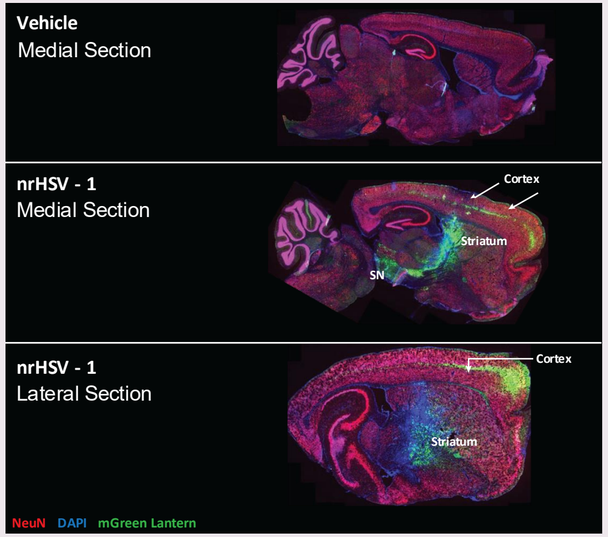

Figure 3 | Mouse brain sections at 1 week after vehicle or EG143A administration immunostained for mGreen lantern (green), NeuN (red) and DAPI (blue). White arrows shows neurons in the cortex expressing mGreen lantern. SN: Substantia nigra.

2 nrHSV-1 transduces exclusively neurons in the cortex

Double immunostaining of mGreen lantern with the neuronal marker NeuN showed that only neurons were transduced by the nrHSV-1 vector in the cortex (Figures 4 and 5).

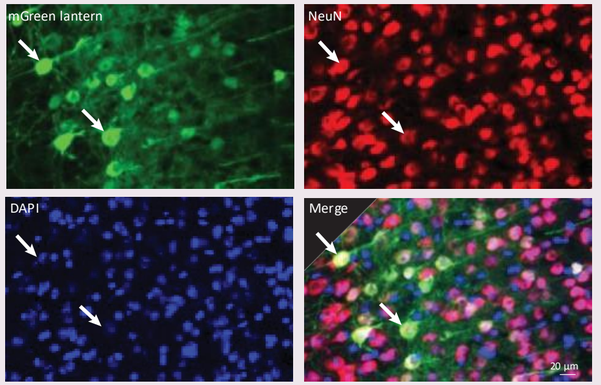

Figure 4 | Mouse brain sections at 1 week after vehicle or EG143A administration immunostained for mGreen lantern (green), NeuN (red) and DAPI (blue). White arrows show colocalization between NeuN and mGreen lantern.

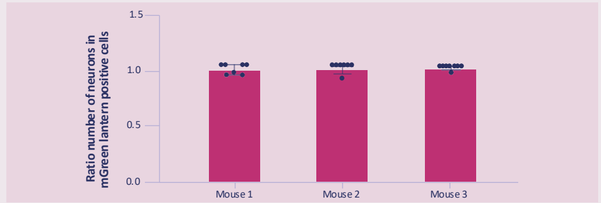

Figure 5 | Quantification of the proportion of neurons among mGreen lantern expressing cells in cortex at one week. Each dot represent one tissue section

3 nrHSV-1 administration induces stable transgene expression in cortical neurons

Quantification of the number of neurons expressing mGreen lantern at 1 and 6 weeks after intrastriatal administration showed similar cell densities (Figure 6A,B). Quantification by ddPCR of vector episomes and mGreen lantern transcripts at 1 and 6 weeks showed stable levels as well (Figure 6C,D).

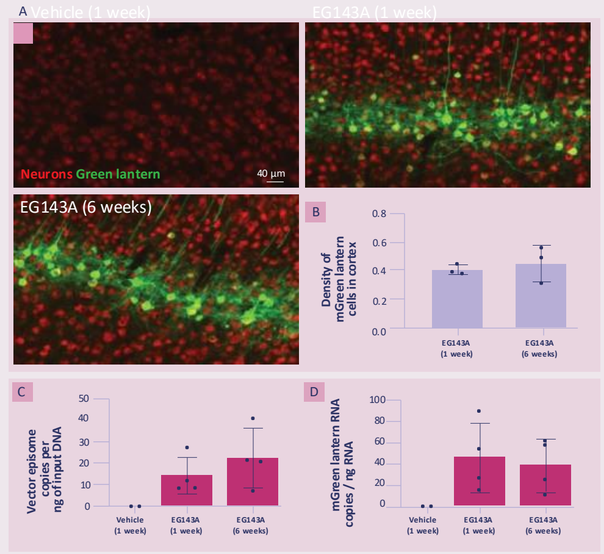

Figure 6 | Long term transgene expression in cortical neurons. A. Cortical sections from mice injected with vehicle or EG143A at 1 and 6 weeks stained for mGreen lantern transgene (green) and the neuronal marker NeuN (Red). B. Quantification of the density of neurons positive for mGreen lantern in cortex at one and 6 weeks. C. Quantification of vector episomes in cortex by ddPCR at one and 6 weeks. D. Quantification of mGreen lantern RNA transcripts in cortex by reverse transcription ddPCR. Mean +/- SD

Conclusion

Our results suggest that intra-striatal administration of low amount of nrHSV-1 vector broadly targets cortical neurons by retrograde transport achieving stable long-term transgene expression for at least 6 weeks. This vector is a tool for gene therapies targeting cortical neurons for the treatment of diseases of the CNS (such as Parkinson's disease, Huntington's disease, or other neurodegenerative diseases with cognitive impairment).

Disclosures: This work was funded by EG 427. All listed EG 427 authors are current employees of EG