A53T alpha-synuclein promotes a rapid neurodegeneration and motor deficit following AAV vector mediated expression in the rat substantia nigra

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Introduction

Adeno-associated viral (AAV) vector mediated expression of alpha-synuclein in different forms provides a means for studying alpha-synuclein-induced motor deficits and pathology in animal models[1,2]. Due to the variation in the serotypes and promoters used, the titre of the AAV vector injected and if the gene is an overexpression of the wildtype (WT) or mutated form of alpha-synuclein (A53T), reports to date differ in terms of emergent motor deficits and neurodegeneration.

Aims

In this study the effects of AAV5-CBA-alpha-synuclein (WT) and AAV1/2-CMV-A53T-alpha-synuclein were assessed in a rat model of synucleinopathy in the substantia nigra (SN) in rats. AAV empty-vector, AAV enhanced green-fluorescent-protein (eGFP) and phosphate buffered saline (PBS) were included as controls.

Methods

Adult male and female Wistar rats received a unilateral stereotaxic injection into the SN. A test of motor behaviour was performed and vector-mediated expression of alpha-synuclein and immunofluorescent analysis of the integrity of neuronal dopaminergic cells in the SN were assessed post-mortem.

Table 1: Virus titre, injection volumes and coordinates for unilateral injections into the substantia nigra.

Treatment groups, Virus titre and Injection volume	Stereotaxic co-ordinates
•Sterile PBS (2µl) •AAV5-CBA-eGFP (2µl; 9.5x10^{12}vg/ml) •AAV5-CBA-alpha-synuclein human WT (2µl; 1x10^{13}vg/ml) Vectors were obtained from Viral Vector Core of the University of Iowa, Iowa City, United States.	Male: AP -5.3mm from bregma ML + 2.0mm from bregma DV -8.5mm from skull
•Sterile PBS (2µl) •AAV1/2-CMV-A53T alpha-synuclein (human) (2µl; >5x10^{12} vg/ml) •AAV1/2-CMV-Null/Empty (2µl; >5x10^{12} vg/ml) •AAV1/2-CAG-miR scrambled control co-expressing eGFP (2µl; >0.5x10^{12} vg/ml) Vectors were obtained from Vigene Biosciences , Rockville, MD, United States.	Male: AP -5.3mm from bregma ML +2.0mm from bregma DV – 8.5mm from skull Female: AP -5.3mm from bregma ML +2.0mm from bregma DV – 7.5mm from skull

The Cylinder motor test was conducted as a measure of forelimb use asymmetry. Rats were placed in an empty glass cylinder for 5 minutes. The number of wall placements made with the contralateral forelimb are expressed as a % of the total number of wall placements made.

Immunofluorescent imaging: Double fluorescence immunostaining was performed on free-floating SN sections using antibodies raised against tyrosine hydroxylase (TH) (mouse, 1:2000, Millipore) as a marker of dopaminergic neurons, and alpha-synuclein (rabbit, 1:2000, Millipore). Images were taken using an AxioImager Z1 epifluorescent microscope with a Zeiss AxioCam HR camera at 10X magnification and an Olympus Ix81 fluorescence microscope and an Andor iXon+ ultra sensitive EMCCD camera at 20X magnification.

Experimental design:
AAV5 alpha-synuclein (WT): behaviour in the cylinder test was assessed 9 and 11 weeks post AAV5 delivery. Animals were transcardially perfused at 12 weeks for tissue collection and pathological assessments

AAV1/2 alpha-synuclein (A53T): In separate experiments behaviour in the cylinder test was assessed 4 and 9 weeks post AAV1/2 delivery. Animals were transcardially perfused at 4 and 12 weeks respectively for tissue collection and pathological assessments.

References

[1]Kirik, D., Rosenblad, C., Burger, C., Lundberg, C., Johansen, T.E., Muzyczka, N., Mandel, R.J., Bjorklund, A. 2002. Parkinson-like neurodegeneration induced by targeted overexpression of alpha-synuclein in the nigrostriatal system. Journal of Neuroscience 22, 2780-2791.
[2]Decressac, M., Mattson, B., Lundblad, M., Weikop, P., Bjorklund, A. 2012. Progressive neurodegenerative and behavioural changes induced by AAV-mediated overexpression of alpha-synuclein in midbrain dopamine neurons. Neurobiology of Disease 45, 939-953.

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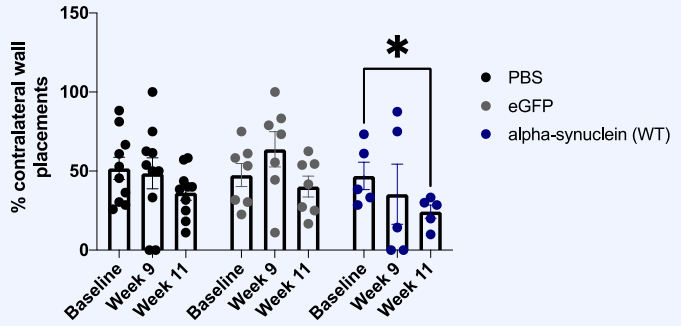
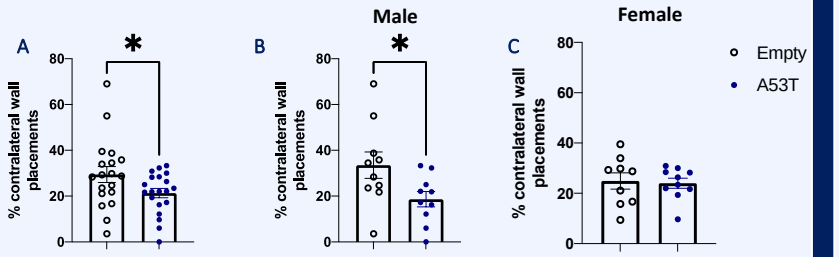
Results

Limb use asymmetry in the cylinder test following AAV5-CBA-alpha-synuclein delivery



There was a decrease in contralateral paw placements in the AAV5 WT group when compared to baseline performance. Data is expressed as mean ± SEM. (N = 5-10 per group; * p <0.05, Two-way repeated measures ANOVA followed by Tukey's multiple comparisons test).

Limb use asymmetry in the cylinder test 9 weeks after AAV1/2-CMV-A53T delivery



There was a decrease in contralateral paw placements in the AAV1/2 A53T group 9 weeks following AAV delivery when compared to empty vector (A). Data presented as mean ± SEM. (N = 19-20 per group, * p <0.05, unpaired t-test). Differences were also identified between groups were observed 4 weeks post AAV delivery (data not shown). When data were analysed for male and female separately, lower contralateral paw placements in the A53T group was observed in males only when compared with empty vector (B), with no differences observed in the females (C) at 9 weeks post AAV delivery. Data presented as mean ± SEM (N = 9-10 per group, * p < 0.05, unpaired t-test).

Conclusions

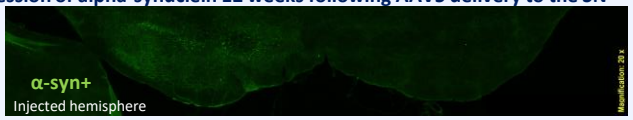
AAV1/2-CMV-A53T alpha-synuclein provoked limb use asymmetry 9 weeks post AAV delivery. A similar reduction was observed with AAV5-CBA-WT 11, but not 9, weeks post AAV delivery.
Both AAV5-CBA-WT and AAV1/2-CMV-A53T lead to alpha-synuclein over-expression in the injected hemisphere of the SN 12 weeks following AAV delivery.
AAV1/2-CMV-A53T shows reduction in TH+ immunofluorescence in the SN 12 weeks following AAV delivery.

Analyses are ongoing to determine differences between treatment groups. Taken together, the data presented here support the use of AAV to produce models to inform research into the utility and reproducibility of AAV based rodent models of alpha-synucleinopathy. These models may subsequently be used to test prospective therapeutics targeting alpha-synuclein.

For more information as well as further details on new projects in development please contact aharkin@tcd.ie

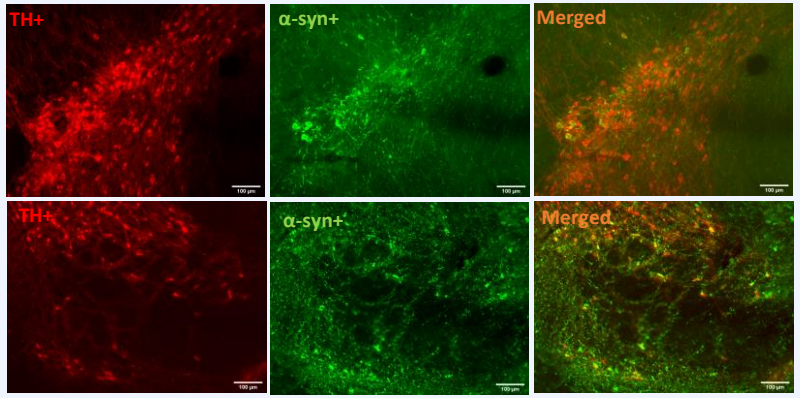
Results

Expression of alpha-synuclein 12 weeks following AAV5 delivery to the SN



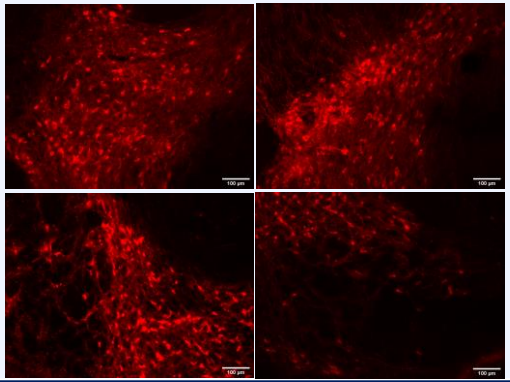
AAV5-CBA-alpha-synuclein WT leads to expression and immunofluorescence of alpha-synuclein (green) in the injected hemisphere of the SN at 12 weeks post-AAV5 delivery.

Expression of alpha-synuclein and its colocalization with TH in the SN of the AAV1/2 injected hemisphere



AAV1/2-CMV-A53T leads to expression and immunofluorescence of alpha-synuclein (green), TH+ (red) and their colocalization (orange) in the injected hemisphere 4 weeks (top panels) and 12 weeks (bottom panels) post AAV1/2 delivery.

TH+ Immunofluorescence 4 and 12 weeks following AAV1/2 delivery to the SN in the injected and non injected hemispheres



Representative TH+ immunofluorescence in the SN in the non-injected hemisphere (top left) and injected hemisphere (top right) 4 weeks post AAV1/2 A53T alpha-synuclein delivery.

Representative TH+ immunofluorescence in the SN in the non-injected hemisphere (bottom left) and injected hemisphere (bottom right) 12 weeks post AAV1/2 A53T alpha-synuclein delivery.