# Does α-synuclein Increase Cell Damage and Inflammation in Neurons and Microglia?

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## Introduction

Parkinson's disease (PD) is a neurodegenerative disease caused by the accumulation of an abnormal protein called α-synuclein. Most PD cases are sporadic, or random, and caused by wild-type (WT) α-synuclein. However, 5-10% of patients are diagnosed with the heritable form of this disease caused by a specific mutation such as A53T in the α-synuclein gene.

Neurons in the brain are not independent, but instead work together with neighbouring support cells such as microglia. Our hypothesis is that α-synuclein over-expression in neurons might trigger microglia to produce inflammation. This could induce further damage in neurons eventually leading to neuronal death (1).

Understanding the role of inflammation on the disease progression of PD may be useful in designing new treatments for PD in the future.

## Aims

This project aims:

- to model the interaction between neuronal and microglia-like cells in a co-culture system similar to the conditions inside the brain
- to investigate the effects of neuronal α-synuclein over-expression on DNA damage and inflammation markers in neuronal and microglia-like cells

## Methods

1. 3 cell lines of human SH-SY5Y neuroblastoma cells were differentiated into neuronal cells:

- **EGFP –** cells transfected with EGFP green fluorescent protein only as control
- **WT –** cells transfected with wild-type α-synuclein fused to EGFP green fluorescent protein
- **A53T –** cells transfected with A53T-mutated α-synuclein fused to EGFP green fluorescent protein

2. Human U937 leukaemia cells were differentiated into microglia-like cells.
3. U937 cells were co-cultured with SH-SY5Y cells. Cell culture inserts were used in several wells to physically separate the U937 and SH-SY5Y cells while allowing some of their media contents to interact (Figure 1). SH-SY5Y cells in other wells were treated with U937 conditioned medium only without the U937 cells.

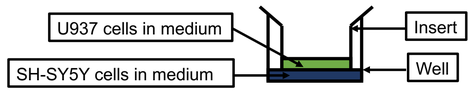
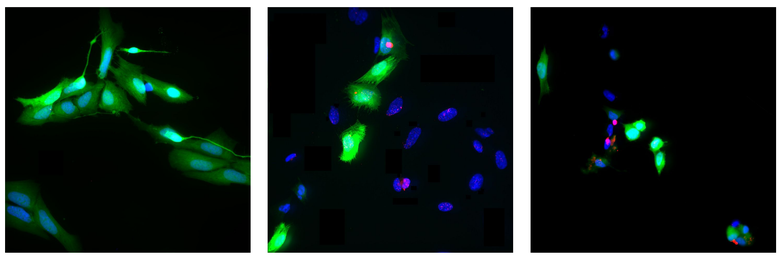



**Figure 1:** Co-culture system using a cell culture insert

4. After co-culture, SH-SY5Y cells were stained with an immuno-staining method to detect DNA damage. ImageJ was used to count the number of cell nuclei with red foci (Figure 2).



A. EGFP SH-SY5Y cells B. WT SH-SY5Y cells C. A53T SH-SY5Y cells

**Figure 2:** Example of image analysed using ImageJ. Blue areas indicate cell nuclei. Red foci indicate DNA damage. Green fluorescence overlapping blue areas indicate transfected cells.

5. Expression of inflammation markers such as TNF-α, COX-2, IL-6 and IL-1β were quantified using quantitative polymerase chain reaction method (qPCR).

## Results and discussion: Cell damage

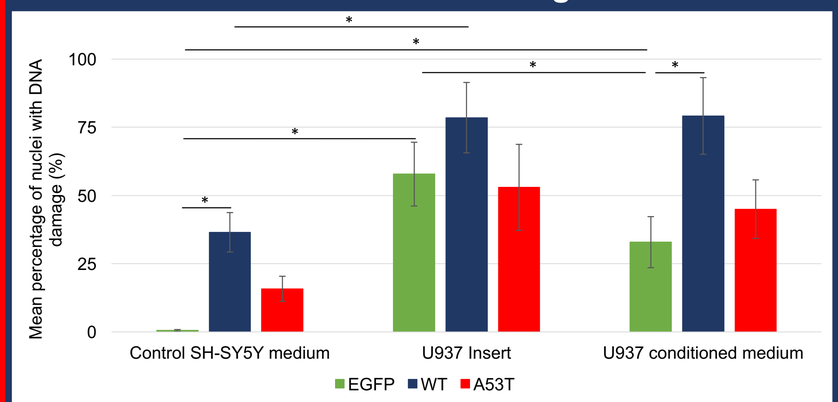



**Figure 3:** Mean percentage of transfected SH-SY5Y cells with nuclei containing DNA damage. * denotes p<0.05

- In control medium and U937 conditioned medium, SH-SY5Y cells containing WT α-synuclein have a significantly higher amount of DNA damage than SH-SY5Y cells expressing EGFP only
- SH-SY5Y cells expressing EGFP only have a significantly higher amount of DNA damage when co-cultured with U937 cells on inserts and when treated with U937 conditioned medium compared to control medium
- SH-SY5Y cells containing WT α-synuclein have a significantly higher amount of DNA damage when co-cultured with U937 cells on inserts compared to when treated with control medium

## Results and discussion: Inflammation markers

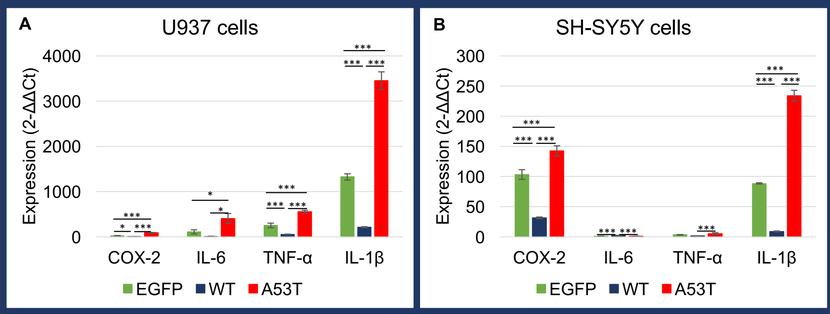



**Figure 4A and 4B:** Expression of inflammation markers in U937 and SH-SY5Y cells in co-culture. * denotes p<0.05. *** denotes p<0.001.

**4A: U937 microglia-like cells**

- For COX-2, TNF-α and IL-1β, U937 cells express a significantly lower amount when co-cultured with SH-SY5Y cells containing WT α-synuclein but a significantly higher amount when co-cultured with SH-SY5Y cells containing A53T α-synuclein

**4B: SH-SY5Y neuronal cells**

- For COX-2 and IL-1β, SH-SY5Y cells containing WT α-synuclein express a significantly lower amount but SH-SY5Y cells containing A53T α-synuclein express a significantly higher amount when co-cultured with U937 cells
- SH-SY5Y cells containing WT α-synuclein express a significantly higher amount of IL-6 compared to EGFP only and A53T α-synuclein when co-cultured with U937 cells

## Conclusion

- Neurons with wild-type α-synuclein are more sensitive to DNA damage in the presence of microglia cells compared to neurons without α-synuclein and neurons with A53T-mutated α-synuclein
- Low levels of inflammation markers are induced by microglia and neurons with wild-type α-synuclein on each other
- High levels of inflammation markers are induced by microglia and neurons with A53T-mutated α-synuclein on each other

## Acknowledgements

I would like to extend my heartfelt gratitude to my supervisor, Dr Gabriele Saretzki, and her team for their guidance and support throughout this project. I would like to thank Newcastle University for providing me with the opportunity to contribute in research in Parkinson's disease.

Key:
p<0.05, significant difference; p<0.001, very significant difference; TNF-α, Tumour necrosis factor alpha; COX-2, Cyclooxygenase-2; IL-6, Interleukin-6; IL-1β, Interleukin-1β

Reference:
1. Rocha, E.M., et al., Alpha-synuclein: Pathology, mitochondrial dysfunction and neuroinflammation in Parkinson's disease, Neurobiol. Dis. (2017), http://dx.doi.org/10.1016/j.nbd.2017.04.004