**α-syn PFF Triggers Stress Responses in Human Astrocytes That Induce Senescence**

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### Introduction

The substantia nigra pars compacta (SNpc) is a structure of the midbrain that is crucial for modulating the initiation of motor movement, among other specific cognitive and emotion-processing functions. SNpc is composed of a group of neurons that fire rhythmically at a rate of 2–10 Hz. This characteristic makes SNpc vulnerable to metabolic stress (Ni et al., 2022; Lin et al., 2021). Moreover, the rhythmic activity of dopaminergic neurons is altered in Parkinson’s Disease (PD) patients and the abnormalities seem to be related to α-synuclein (Bove et al., 2019). In normal conditions, astrocytes sustain neuronal function but what happens if they become toxic or unresponsive? Recently, it has been observed that there is a wide spectrum of phenotypes that astrocytes may acquire depending on the signaling they encounter (Escartín et al., 2021). However, the most striking observation is that astrocytes become progressively impaired under protein-misfolded pathological conditions (Zimmer et al., 2024). We hypothesize that astrocytes exposed to neurodegeneration neurons that leave behind α-syn aggregates become reactive and Mar2-1 revert this status. Mar1-1 synthesis is linked to the changes observed in the phenotype of astrocytes (Fig 1). Here we uncover a novel reactivity acquired by astrocytes exposed to α-syn-PFF characterized by the expression of senescence and stress markers along with the induction of the NFκB nuclear translocation.

### Methods

- **Human and rat astrocyte culture:** primary cultures of astrocytes (Cell Applications Inc., San Diego, CA) were culture following media and directions provided by distributors. Briefly, rat and astrocyte cultures were thawed and plated at passage 2 and expanded up to passage 5. The cells were plated and treated as described in each figure.
- **Immunocytochemistry:** Immunostaining took place as follows: cells were fixed with 4% paraformaldehyde solution for 20 min, washed with PBS and permeabilized with 0.1% Triton-X. After blocking with 1% BSA and 10% Donkey normal serum, primary culture was added overnight in humid chambers at 4°C. The cells were washed and incubated for one hour with secondary antibody conjugated with Alexa-fluor 555, DAPI (Thermo Fisher cat# DJ306) was used for nuclear, and cell mask (Thermo Fisher cat# C10046) for cell membrane staining. Z-stacks were obtained for 5 random fields in a Fluoview 3000 laser confocal microscope. Images were converted to and processed using IMARIS 9.9 using the Cell module.
- **Real-Time PCR:** Cells were scrapped using RT buffer and processed for total RNA extraction using RNAeasy plus kit (Qiagen, Germantown, MD). Total RNA was measured using Nanodrop. First strand synthesis of cDNA was performed using iScript Mastermix cDNA synthesis kit (BioRad, Hercules CA). Real-Time PCR was performed using Taq-Man pre-design primers from LifeTechnologies (ThermoFisher, Whaltham, MA).
- **Statistical analysis:** q-PCR CT were processed by the deltaCT method. Plots and statistical treatment were performed using GraphPad 10.0. The data was first analyzed using one-way analysis of variance (ANOVA) and multiple comparisons via Tukey’s honest significant differences test. The pairwise comparison was performed using a two-tailed Student’s t-test.

### Results

![Figure 2](image1.png)

![Figure 3](image2.png)

![Figure 4](image3.png)

### Conclusion

1. Mar-1 decreased the activation of NFκB/p65 by α-syn PFF (Fig. 2).
2. α-syn PFF induced the upregulation of the senescence marker CDKN2B/p15INK4b (Fig. 3).
3. the increase transcription of HMGB1 and IL1B (Fig. 3).
4. Mar-1 counteracted the effect of Mar-1 on the transcription of stress, inflammation, and senescence markers.
5. The activity of ALOX12 was decreased by α-syn PFF (Fig. 4).

Overall, α-syn PFF disabled astrocytes to respond to cues by induce senescence and Mar-1 prevent this change of phenotype.

### Future Direction

In future directions we will focus on the mechanisms by which α-syn PFF induces senescence and the link between this cellular process and the decrease in the synthesis of Mar-1.

### References

Ni A, Ernst C. Evidence That Substantia Nigra Pars Compacta Dopaminergic Neurons Are Selectively Vulnerable To Oxidative Stress Because They Are Highly Metabolically Active. Front Cell Neurosci. 2022;16:826193. PMCID: PMC691026


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