“Maresin-1 protects Dopaminergic neurons in the Sustantia Nigra in a rat model using misfolded alpha-synuclein seeding.”

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Introduction

Parkinson's Disease (PD) is a neurodegenerative disease affecting over 11.77 million people in 2021 (Luo et al, 2021) and is characterized by the loss of dopaminergic neurons found in the Sustancia Nigra pars compacta (SNpc). These dopaminergic neurons serve the purpose of conducting movement, emotional processing, and other cognitive functions. As of today, there are no known cures for PD, but there are treatments that helps the neurotransmission of dopamine and to induce relief of the motor symptoms. It is vital to treat PD before the onset of motor symptoms because at that point, 60-70% of cells are dead (Cheng, 2010). To resolve this, a general timeline must be produced to determine when early symptoms occur. One hallmark of PD is olfactory dysfunction that can happen years before motor symptoms take place (Ponsen, 2009). In addition, we hypothesize that Maresin-1 (Mar-1), an omega-3 derivative that has been shown to have anti-inflammatory properties in cells, can be used to impair the formation of α-Synuclein (α-Syn) in Lewy bodies (LB). α-Syn are misfolded protein aggregates that are detrimental to a neuron and cause it to die. In this procedure, we discover the effectiveness of Mar-1 on α-Syn –PFF and produce a timeline of when olfactory impairment occurs.

Methods and Materials

- **α-syn Preformed Fibrils (PFF) seeding via intranasal route and Maresin-1 (Mar-1) treatment**: we administered 60 mg of PFF in 12 ul (30 ug per nostril) or saline (control). The following 2 weeks, two doses of 5 mg of Maresin-1 (2.5 ug per nostril) or the equivalent volume of saline was delivered. For this purpose, we use light anesthesia (isoflurane by inhalation route in a mix of oxygen and nitrous oxide) using a vaporizer. Three groups of five rats were used to test olfaction deficit via buried food test from weeks 22 to 26 after initial treatment in two different cohorts of male rats. After 40 weeks rats were perfused using Formalin and brain tissue was dissected.
- **Behavioral procedures**: the rats were housed separately and prepared for Buried food test (BFT). Briefly, in BFTs, rats were trained to eat Fruit Loops (Kellogg Cereals) for 3 weeks before testing. Under red light, rats were allowed to find the food hidden under the bedding, and the time spanning until they found the Fruit Loop was recorded. After 600 second when the rats did not find the food, the testing was stop and it was registered that time mark. The testing proceeded from week 22 to week 26. In both cohorts the first failure was registered in week 24. Results were compared for each week using one-way ANOVA and multiple comparisons test, Tukey’s HSD.
- **Congo red staining**: The fixed brains were sliced into 50 mm thick and delipidated using increasing concentrations of ethanol (70%, 80%, 90%, 95% and 100%) and then rehydrated by reversing the concentrations. The tissue was immersed in Congo Red solution for one hours and then rinsed three times in Ethanol 70%. After stabilization in PBS, Hoechst staining was applied. The brain slices were imaged using an emission filter for Texas red in a laser confocal microscope. The Z-stacks were analyzed with the surface module of Imaris 10.2.

Results

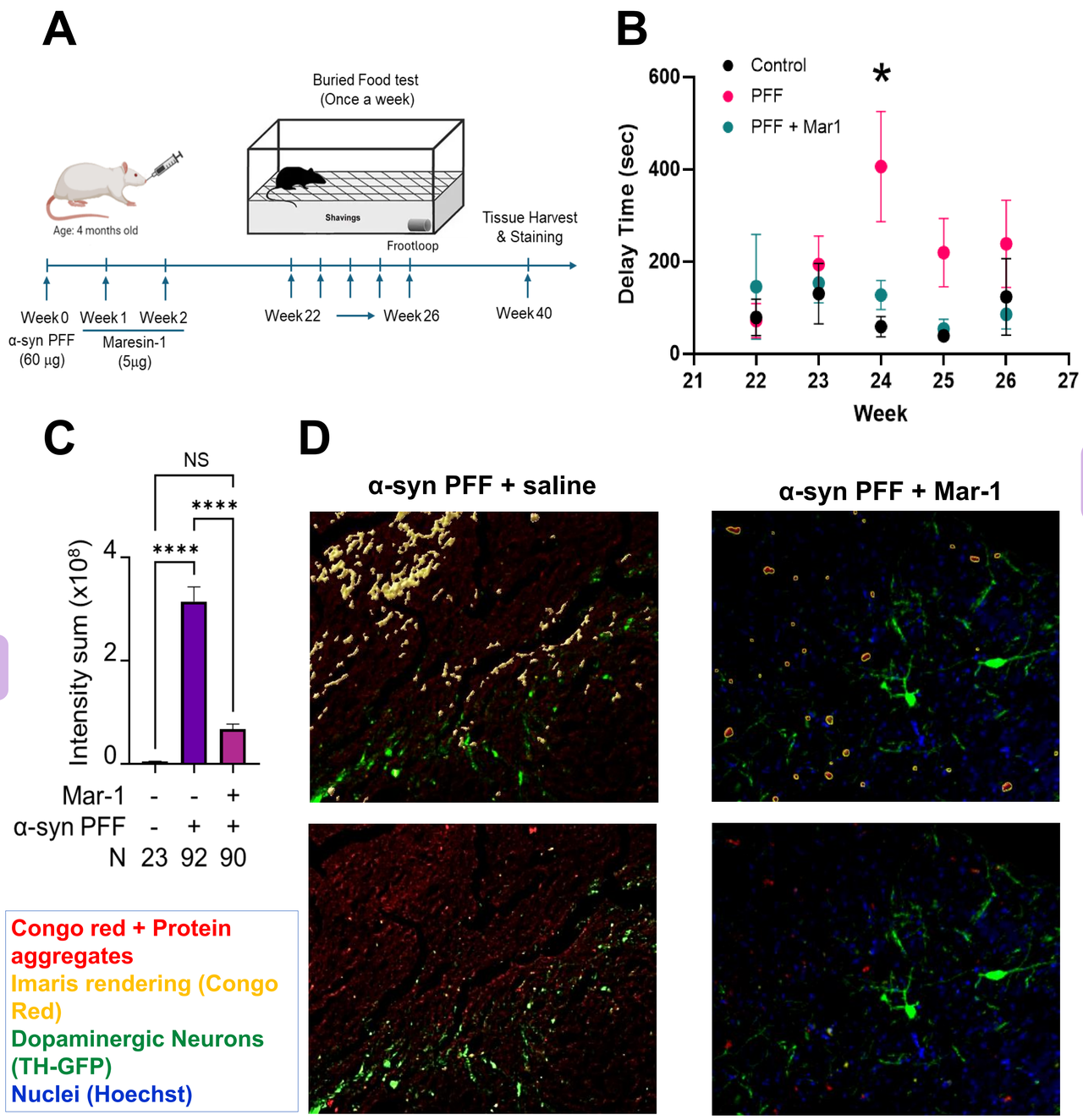

Figure 1

Figure 1. Accumulation of α-Syn-PFF in SNpc. A. Timeline leading to olfaction disfunction after twenty-four weeks. Rats were administered 60 mg of PFF in 12 ul (30 ug per nostril) or saline (control) and were used to test olfaction impairment via BFT from weeks 22 to 26 after initial treatment in two different cohorts of male rats. After 40 weeks, the rats were perfused Formalin, and the brain tissue was harvested and used for staining. **B. Weekly BFTs determine both cohorts had olfaction disfunction at the twenty-fourth week.** Results were compared for each week using one-way ANOVA and multiple comparisons test, Tukey’s HSD. Control vs. PFF: p=p.001; PFF vs. PFF + Mar-1: p=0.01; Control vs. PFF + Mar-1; p=0.9853. **C. Mar-1 administration decreases the number of aggregates found in neurons.** Administration of Mar-1 to tissues with PFF is shown to decrease inflammatory properties of neurons, thus preserving their lifespan. The graph indicates a major significance in the amount of protein aggregates found in tissue with Mar-1 + PFF and tissue with only PFF. **D. Congo red staining reveals Mar-1 reduces the amount of protein aggregates in tissues administered PFF.** Tissue from the SNpc were sliced into 50mm thick and stained with Congo Red. The red indicates the number of aggregates found in the tissue, yellow being the Imaris rendering, the green indicating dopaminergic neurons, and blue representing the nuclei.

Conclusions

- Maresin-1 has shown to reduce the amount of α-Syn protein aggregates.
- Loss of olfactory senses occurs around the twenty-fourth week after exposure to α-Syn-PFF.

Overall, Maresin-1 has a significant impact on the production of α-Syn aggregates and loss of olfaction has a connection with the accumulation of α-Syn.

Future Direction

In the future experiments, we will determine the astrocyte implications in the mechanisms leading to the death of the dopaminergic neurons in SNpc and we will investigate the effects of MaR-1 on neurons and astrocytes during the early stages of PD.

References

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Acknowledgments

Genetics summer program, Neuroscience SUN program

This research project was supported through the LSU Health Sciences Center, School of Medicine.