Nanovesicles as 'Trojan Horses' in Brain Disorders with a Focus on Multiple System Atrophy

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Introduction

- α-Synucleinopathies encompass Parkinson's disease (PD), Parkinson's disease dementia (PDD), multiple system atrophy (MSA) and dementia with Lewy Bodies (DLB).
- The common molecular hallmark of these diseases is the aggregation of the presynaptic neuronal protein α-Synuclein (αSyn).

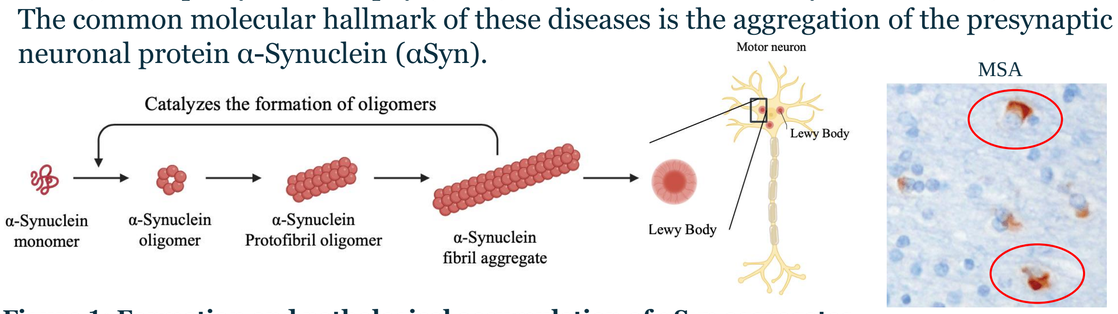


Figure 1: Formation and pathological accumulation of αSyn aggregates. The misfolding of αSyn monomers leads to the formation of oligomers, which assemble into protofibrils and ultimately into fibrillar aggregates. These aggregates contribute to the formation of Lewy bodies within neurons. The image on the right shows immunohistochemical staining of brain tissue under MSA diagnosis, highlighting αSyn-positive inclusions (red circles), a pathological hallmark of Synucleinopathies.

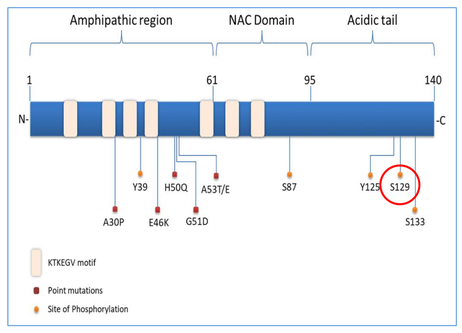
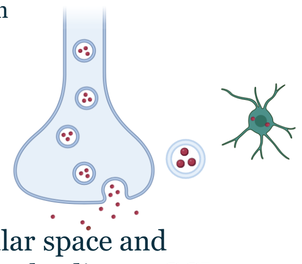


Figure 2: Structure of αSyn protein. The N-terminal is characterized by an abundance of lipid-binding domains and mutation sites, whereas the C-terminal by phosphorylation sites (e.g. Ser129). The NAC region encompasses non-polar side chains involved in fibril formation and protein aggregation



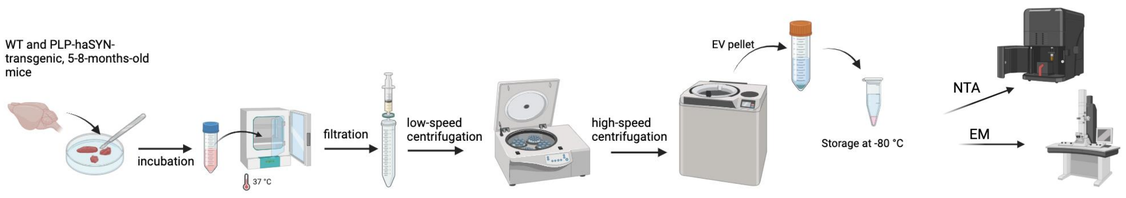
- Pathological αSyn is released from neurons into the extracellular space and taken up by neighboring oligodendrocytes through endocytosis, leading to GCI formation and microglial activation through the release of pro-inflammatory cytokines.

Objectives

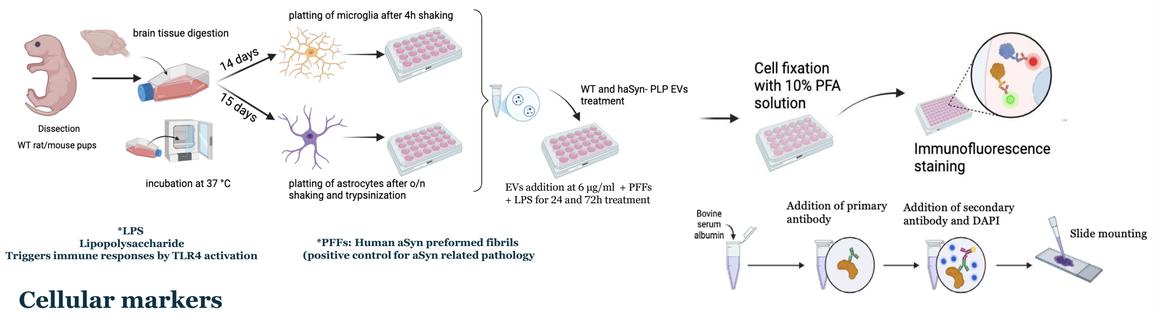
- Elucidate the mechanisms of αSyn transmission and hypothesize that αSyn released by extracellular vesicles may evoke effects on recipient cells.
- Isolate brain-derived extracellular vesicles from wild type (WT) mice expressing endogenous basal levels of αSyn or from proteolipid protein (PLP)-human αSyn (haSyn)-transgenic mice, that overexpress human αSyn under the oligodendroglial-specific PLP promoter.
- Understand the impact of brain-derived WT and haSyn-PLP-derived extracellular vesicles in the initiation and propagation of αSyn-related pathology on primary glial cells of the CNS, with an emphasis on microglia and astrocytes..
- Assess the inflammatory responses and analyze potential markers of astroglia and microglia activation following treatment with brain-derived extracellular vesicles.

Methodology

a. Brain-derived extracellular vesicle extraction and analysis through NTA and EM



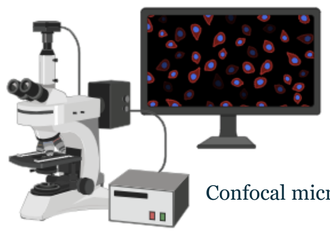
b. Mixed glial cell cultures from WT rat/mouse pups and immunofluorescence assay



Cellular markers
GFAP- Glial Fibrillary Acidic Protein (astrocytes)
IBA1 - Ionized Calcium- Binding Adaptor Molecule (microglia)

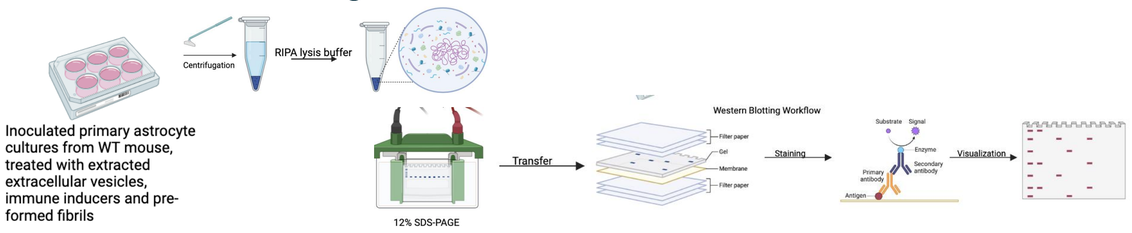
α-Synuclein types/conformations
LB509 - Human αSyn
SYN303- Oxidated/Nitrated αSyn
ROD (D37A6)→ Rodent αSyn

Immune activation markers
MAC2 - Galectin- 3 (microglia activation)
CD45- Leukocyte common antigen (microglia activation)



Confocal microscopy evaluation

c. Western Immunoblotting



Scientific figures were generated through BioRender illustration software.

Results

- **Brain Exosomes as Potential Carriers of Pathological Proteins**

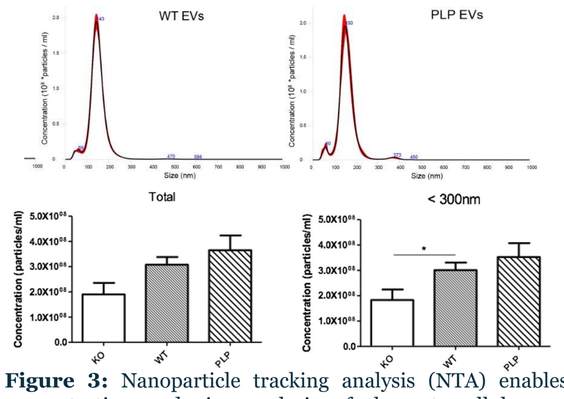


Figure 3: Nanoparticle tracking analysis (NTA) enables the concentration and size analysis of the extracellular vesicles present in sample preparations.

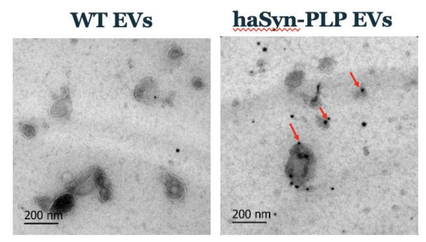
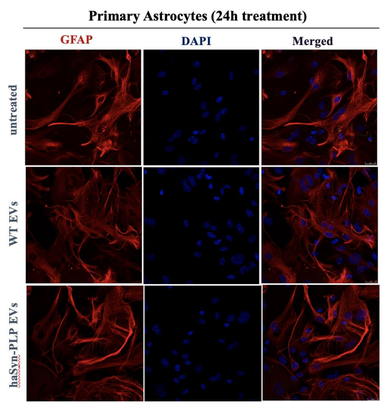
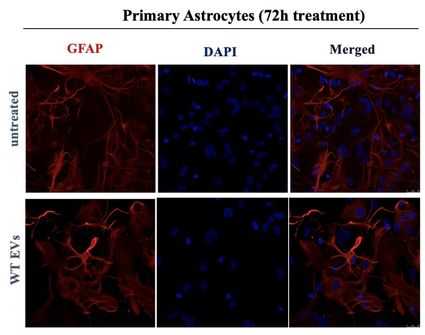


Figure 4: Immuno-EM analysis using an antibody specific for pSer129 aSyn: EVs isolated from PLP mice are enriched with the pathology-related pSer129aSyn.





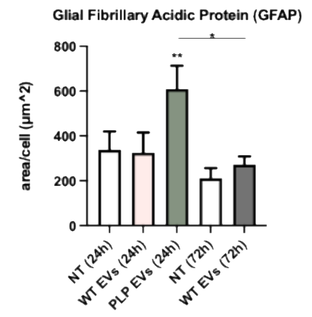
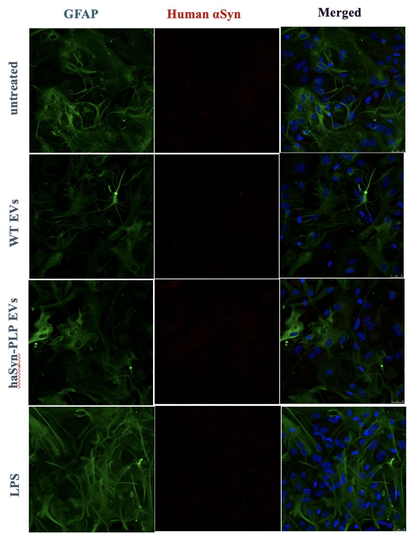
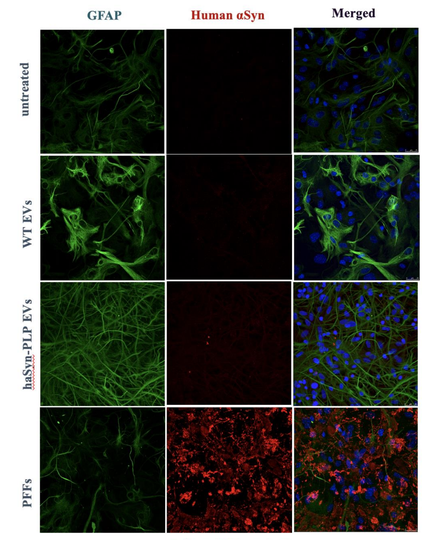


Figure 5: Astrocytes treated with EVs isolated from mouse PLP-haSyn brains showcase higher GFAP levels compared to NT or EVs derived from WT brains, indicating active gliosis.





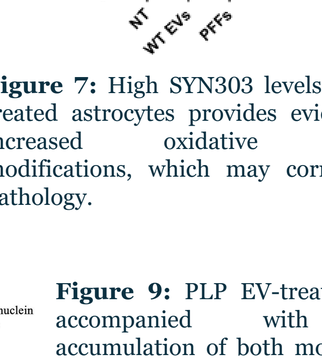


Figure 6: Detection of haSyn in astrocytes treated with haSyn-PLP EVs. Background signal in NT and WT-EV treated cultures.

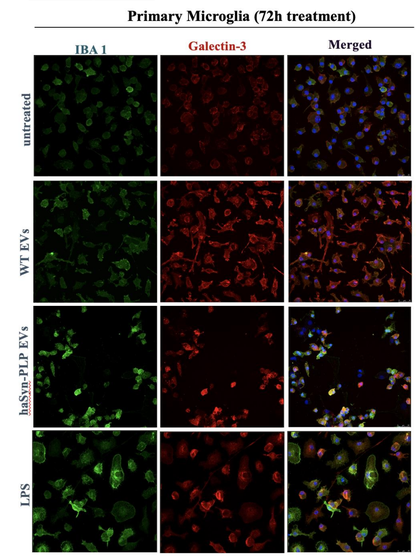
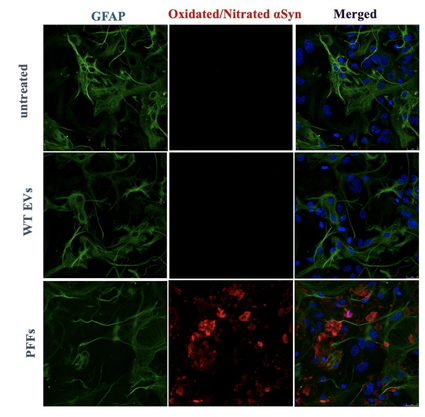


Figure 8: Increased IBA1+area is detected in both WT- and PLP-EVs, indicative of microglia activation. At the same time, higher Galectin-3+ signal in PLP EV-treated microglia further points to an active neuroinflammatory response.



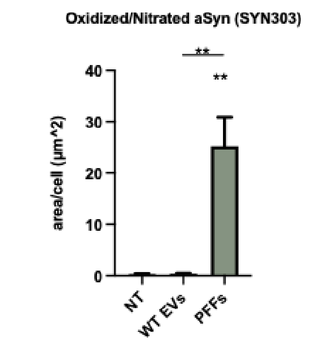


Figure 7: High SYN303 levels in PFF-treated astrocytes provides evidence of increased oxidative protein modifications, which may correlate to pathology.

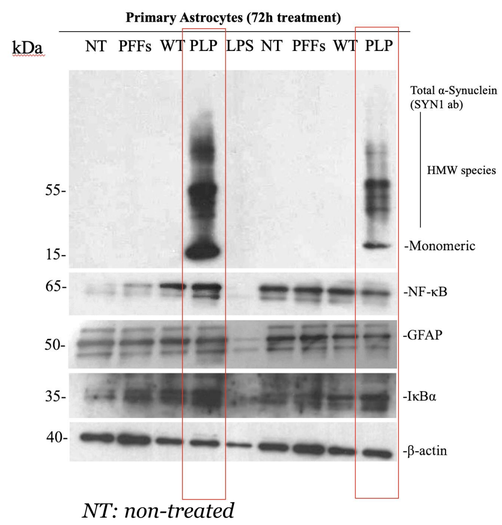


Figure 9: PLP EV-treatment is accompanied with the accumulation of both monomeric and high-molecular weight aggregated aSyn species within primary astrocyte cultures.
At the same time, detection of increased levels of the p65 NF-kB regulatory subunit suggests the presence of active inflammatory response. Interestingly, IκB are also increased, probably indicating an impairment of intracellular proteolytic machineries responsible for IkBa clearance.

NT: non-treated
PFFs: pre-formed αSyn fibrils
WT: EVs isolated from WT mouse brain
PLP: EVs isolated from haSynPLP mouse brain (MSA mouse model)

Conclusion

- Collective data indicate that haSyn-PLP EVs promote the activation of microglia and astrocytes, implicating neuroinflammation and aSyn protein aggregation.
- Detectable signal of human aSyn in haSyn-PLP EV-treated mouse glial cultures suggests effective uptake of human aSyn by healthy, neighboring astrocytes, further highlighting the role of EVs as cargos of pathological proteins.

Acknowledgments

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References

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