Whole-brain 3D quantification of alpha-synuclein spreading in a mouse model of Parkinson's disease

Authors

Frederikke Lynge Sørensen[1,2], **Yasir Gallero-Salas[1]**, Annika Højrup Runegaard Thomsen[1], Urmas Roostalu[1], Helene Ægidius[1], Jacob Lercke Skytte[1], Evi Alexiou[1], Jacob Hecksher-Sørensen[1], Poul Henning Jensen[2], Henrik H Hansen[1].

[1]Gubra, Hørsholm, Denmark
[2]Department of Biomedicine and DANDRITE, Danish Research Institute of Translational Neuroscience, Aarhus University, Denmark.

Corresponding authors
Yasir Gallero-Salas - ygs@gubra.dk
Frederikke Lynge Sørensen – fso@gubra.dk

Background & Aim

Progressive spreading of α-synuclein (αSyn) in combination with changes in the dopaminergic system constitute histopathological hallmarks of Parkinson's disease (PD). While existing preclinical models often recapitulate advanced stages of PD, we here applied 3D whole-brain imaging to provide a combined spatiotemporal map of changes in αSyn aggregate architecture and tyrosine hydroxylase (TH) positive cell populations over the course of synucleinopathy in the αSyn pre-formed fibril (PFF) mouse model of early-stage PD.

Methods

See Figure 1 for study outline. 8-weeks old C57BL/6RJ mice received unilateral stereotaxic injections of murine αSyn PFFs (5 µg per injection site) in the dorsal striatum. Mice receiving intrastriatal injections of αSyn monomer served as controls. Mice were terminated at 1-, 4-, 8-, 12-, 16-, or 26-weeks post-injection (wpi), and whole-brains were immunolabelled for αSyn phosphorylated at serine-129 (pS129-αSyn) and TH, optically cleared (iDISCO+) and scanned using light sheet fluorescence microscopy (LSFM). AI-based computational analysis enabled automated mapping and quantification of pS129-αSyn and TH fluorescence signal across 840 individual brain regions using a custom mouse brain atlas.

Conclusion

+ Using whole-brain imaging and AI, we identified predictable patterns of αSyn spreading from the site of disease onset to anatomically connected brain regions
+ This spread may coincide with not only loss of dopaminergic neurons but also a reduction in their projections
+ The developed 3D imaging and AI analysis pipeline will help in drug discovery research with unbiased an accurate assessment of drug efficacy

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1 Automated whole-brain imaging pipeline

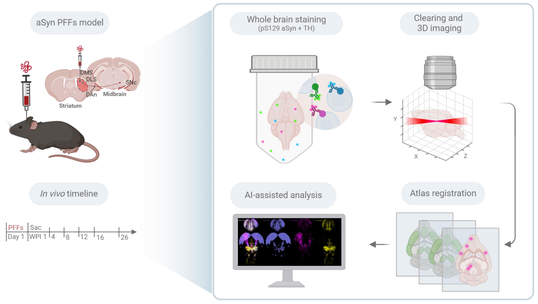

Figure 1. Study design and LSFM workflow. αSyn mPFFs were unilaterally injected into the striatum. Mice were terminated at 1 to 26 wpi, and whole-brains were co-labelled for pS129-αSyn and TH, cleared, and scanned using LSFM. AI-based computational pipeline was applied for automated mapping and quantification of fluorescent signals.

2 Dual staining of pS129/TH

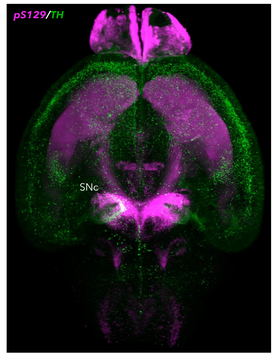

Figure 2. Raw 3D image of whole-brain αSyn+TH labelling (8 wpi). Note pS129-αSyn signal is only visible in the ipsilateral substantia nigra pars compacta (SNc). pS129 staining is shown in green, TH in magenta, and overlapping signal in white.

3 3D maps of αSyn spreading reveals spatiotemporal dynamics

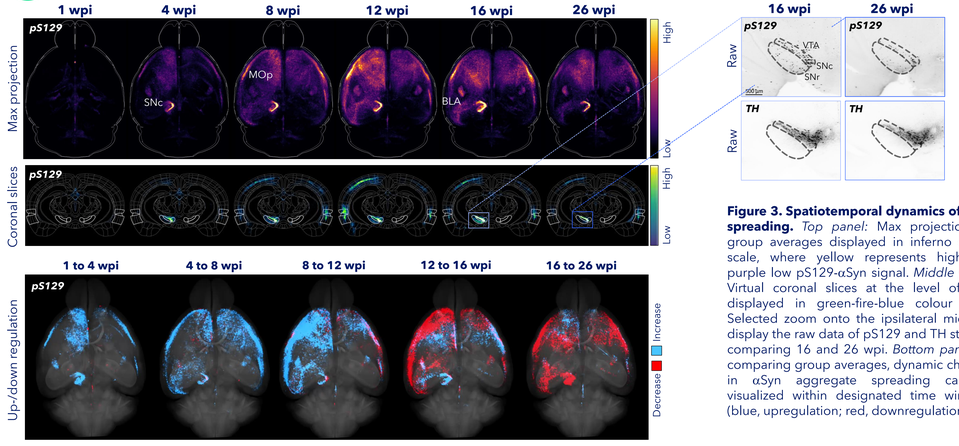

Figure 3. Spatiotemporal dynamics of αSyn spreading. *Top panel:* Max projections of group averages displayed in inferno colour scale, where yellow represents high and purple low pS129-αSyn signal. *Middle panel:* Virtual coronal slices at the level of SNc, displayed in green-fire-blue colour scale. Selected zoom onto the ipsilateral midbrain display the raw data of pS129 and TH staining comparing 16 and 26 wpi. *Bottom panel:* By comparing group averages, dynamic changes in αSyn aggregate spreading can be visualized within designated time windows (blue, upregulation; red, downregulation).

4 Multi-class TH segmentation allows detection of early-stage changes in neuronal projections before cell death occurs

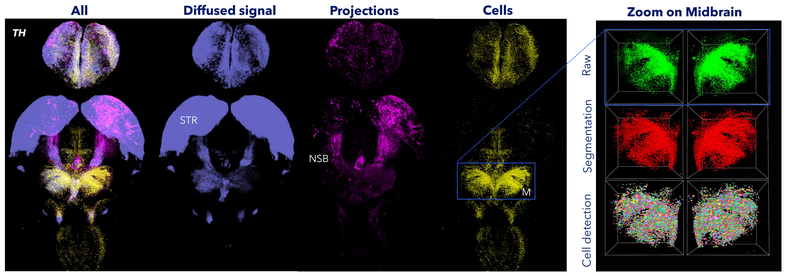

Figure 4. Whole-brain phenotyping approach to map changes in the dopaminergic system in αSyn PFF mice. *Left:* We aim to segment and determine tyrosine hydroxylase (TH)-positive changes in three compartments based on following three classifiers: diffused signal in striatum (STR), neuronal projections in the nigrostriatal bundle (NSB), and neuronal cells in the midbrain (M). *Right:* Preliminary results using 3D Object Analysis (AIVIA) revealed a 13% decrease in TH-positive cells in the ipsilateral side compared to the contralateral side of the midbrain at 12 wpi. Green visualizes the raw TH signal, red the segmented signal, and multicolour individual detected cells.

5 From whole-brain quantification to unbiased region-specific cluster analysis of αSyn spreading

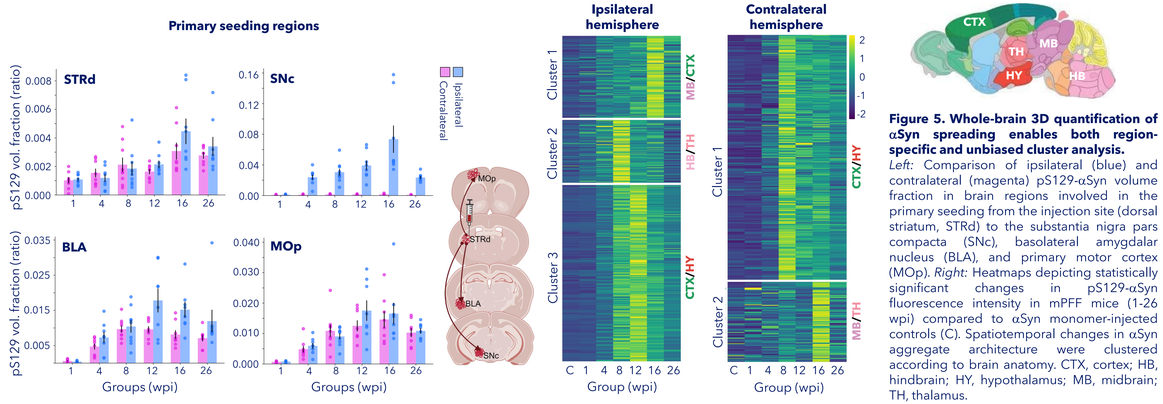

Figure 5. Whole-brain 3D quantification of αSyn spreading enables both region-specific and unbiased cluster analysis. *Left:* Comparison of ipsilateral (blue) and contralateral (magenta) pS129-αSyn volume fraction in brain regions involved in the primary seeding from the injection site (dorsal striatum, STRd) to the substantia nigra pars compacta (SNc), basolateral amygdalar nucleus (BLA), and primary motor cortex (MOp). *Right:* Heatmaps depicting statistically significant changes in pS129-αSyn fluorescence intensity in mPFF mice (1-26 wpi) compared to αSyn monomer-injected controls (C). Spatiotemporal changes in αSyn aggregate architecture were clustered according to brain anatomy. CTX, cortex; HB, hindbrain; HY, hypothalamus; MB, midbrain; TH, thalamus.