Whole-brain 3D mapping and quantification of pathological α-Synuclein aggregate spreading and toxicity in a mouse model of early-stage Parkinson's disease

Authors
Frederikke Lynge Sørensen[1,2], Yasir Gallero-Salas[1], Annika Højrup Runegaard Thomsen[1], Evi Alexiou[1], Martin Rønn Madsen[1], Jacob Lercke Skytte[1], Thomas Topilko[1], Poul Henning Jensen[2], Urmas Roostalu[1], Jacob Hecksher-Sørensen[1], Henrik H. Hansen[1].

[1]Gubra, Hørsholm, Denmark
[2]DANDRITE & Dept. of Biomedicine, Aarhus University, Aarhus, Denmark.

Corresponding author
Frederikke Lynge Sørensen, fso@gubra.dk

Background & Aim

α-Synuclein (α-Syn) aggregate spreading is a neuropathological hallmark of Parkinson's disease (PD). Further mechanistic insight into α-Syn driven pathological events in prodromal PD is essential in target and drug discovery. However, existing preclinical models of synucleinopathy typically portray advanced stages of PD with significant dopaminergic neuronal loss. Using 3D whole-brain imaging, the present study aimed to longitudinally profile α-Syn aggregate architecture in a standard mouse model of synucleinopathy.

Methods

See Figure 1 for study outline. 8-weeks old C57BL/6Rj male mice received a unilateral stereotaxic injection of mouse α-Syn PFFs (mPFFs, #SPR-324, StressMarq Biosciences) in the medial and lateral dorsal striatum (DMS, DLS), respectively (5 µg per injection site). Mice were terminated at 8- or 12-weeks post-injection (WPI, *n*=4 per group). Non-injected mice (*n*=2) served as normal controls. Intact brains were co-immunolabelled for phosphorylated α-Syn (pS129) and tyrosine hydroxylase (TH), optically cleared (iDISCO+) and scanned using light sheet fluorescence microscopy (LSFM) at cellular resolution. An AI-based pipeline was developed for automated whole-brain mapping and quantification of pS129 α-Syn fluorescence intensity in 840 individual brain regions, using a custom mouse brain atlas.

www.gubra.dk

1 Automated whole-brain imaging pipeline

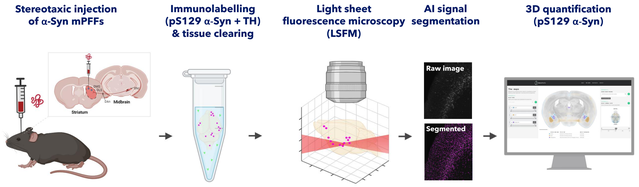

Figure 1. Study design and LSFM workflow. α-Syn mPFFs were unilaterally injected into the striatum. Mice were terminated at 8- and 12-weeks post-injection, respectively. Whole-brains were co-immunolabelled for α-Syn phosphorylated at serine-129 (pS129) and tyrosine hydroxylase (TH), cleared, and scanned using light sheet fluorescence microscopy (LSFM). AI-based computational pipeline was applied for automated anatomical mapping and quantification of fluorescent pS129 α-Syn.

2 Whole-brain α-Syn aggregate & TH architecture

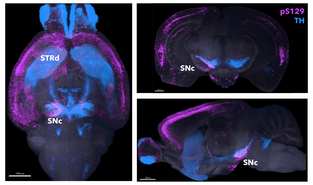

Figure 2. Whole-brain 3D images of α-Syn (pSer129) and tyrosine hydroxylase (TH) expression in the mPFF mouse. Only α-Syn signal is visible in the ipsilateral SNc. pS129 (magenta), TH R667 (blue), tissue autofluorescence (bone) in mPFF mouse (12 WPI). Left panel: Dorsal view (ventral volume). Right upper panel: Coronal view (150 µm). Lower right panel: Sagittal view (500 µm). SNc, substantia nigra compact part; STRd, dorsal striatum.

3 Architectural changes in α-Syn aggregate spreading

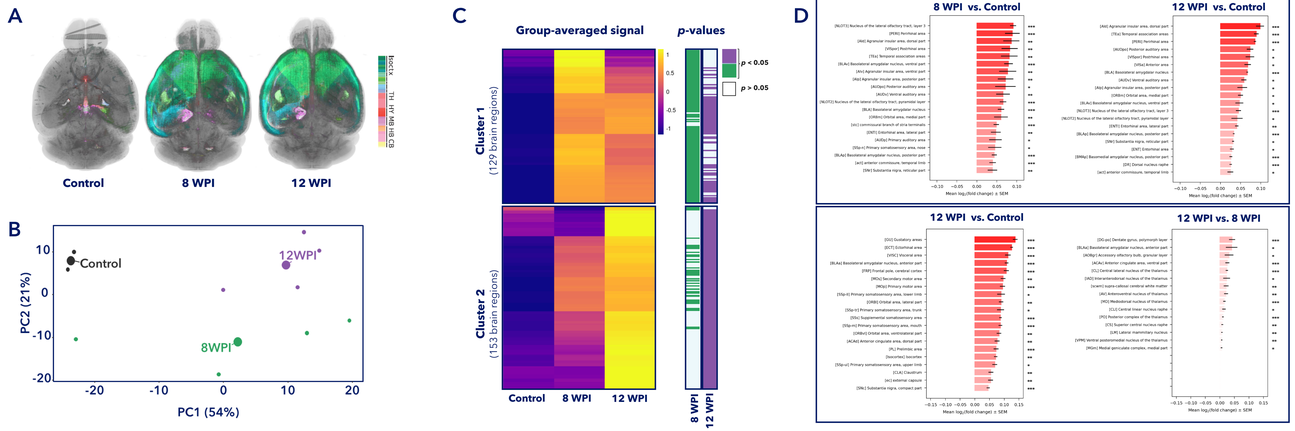

Figure 3. Voxel-based whole-brain analysis of α-Syn aggregate spreading in mPFF mouse model of Parkinson's disease. (A) Whole-brain segmentation of pS129 α-Syn immunofluorescence signal indicates widespread distribution of pathological α-Syn expression in the mPFF mouse in both the ipsilateral and contralateral hemisphere. Whole-brain images illustrate group-average max projections with brain regions color-coded according to the Allen Mouse Brain Reference Atlas, CCFv3. **(B)** Principal component analysis (PCA) of brain samples, depicting the degree of overall variability in study groups. **(C)** Heatmap of a total of 282 individual brain regions with statistically significant change in pS129 α-Syn fluorescence signal, 8 and 12 WPI groups relative to the Control group (p<0.05). Cluster 1 includes brain regions with an early (8 WPI) increase in pS129 signal (predominantly cortical regions), while Cluster 2 represents brain regions with a later increase in signal (12 WPI, predominantly subcortical regions). **(D)** Top-20 brain regions with significant change in pS129 α-Syn expression ranked by log2-fold change compared to the Control group. Upper panel: Cluster 1; lower panel: Cluster 2. *p<0.05, **p<0.01, ***p<0.001 compared to the Control group. WPI, weeks post injection.

4 Anatomical map of α-Syn aggregate spreading

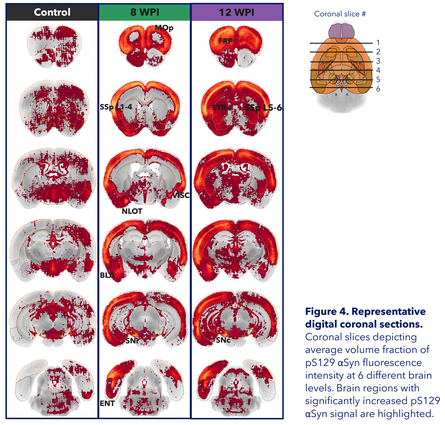

Figure 4. Representative digital coronal sections. Coronal slices depicting average volume fraction of pS129 αSyn fluorescence intensity at 6 different brain levels. Brain regions with significantly increased pS129 αSyn signal are highlighted.

5 α-Syn aggregate distribution in key brain regions affected in Parkinson's disease

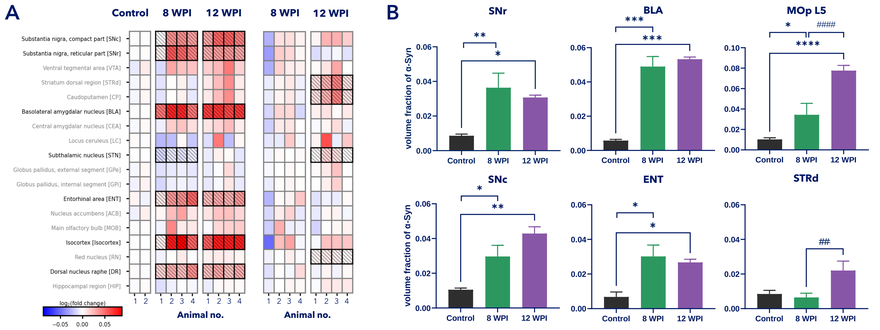

Figure 5. α-Syn aggregate distribution in brain regions known to be involved in PD. (A) Heatmap of pS129 α-Syn-positive voxels (volume fraction) in each animal relative to Control mice (red color, increased; blue color, reduced). Brain regions with statistically significant pS129 αSyn signal compared to Control mice are highlighted in hash and bold frame. **(B)** Selected brain regions in mPFF mice with significantly increased pSer129 α-Syn expression (volume fraction, mean ± S.E.M.) *p<0.05, **p<0.01, ***p<0.001, ***p<0.0001 compared to Control mice, or #p<0.05, #p<0.0001 compared to 8 WPI group (Dunnett's test negative binomial generalized linear model). BLA, basolateral amygdala; ENT, entorhinal cortex; MOp L5, primary motor area, layer 5; SNc, substantia nigra compact part; SNr, substantia nigra reticular part; STRd, dorsal striatum.

Conclusion

- Our AI-based LSFM pipeline can automatically visualize, map, and quantify α-Syn aggregate spreading in the intact mouse brain, including TH-positive dopaminergic brain regions.
- The anatomical complexity of α-Syn aggregate spreading in the PFF mouse model highlights that only whole-brain 3D imaging can fully capture spatiotemporal changes in α-Syn pathology.
- Architectural signatures of early-stage synucleinopathy in the PFF mouse model may potentially be targeted by neuro-protective candidate drugs for PD.

Scan QR code to access the poster online