The Effect of alpha-Synuclein Aggregates on Astrocyte Calcium Signaling in Neurodegeneration

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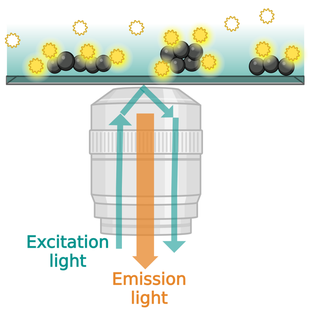
Introduction

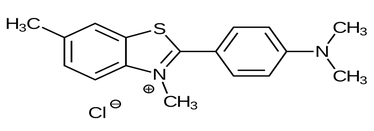
- Parkinson's disease (PD) affects between 1-2 % of the population aged over 65, being the second most common neurodegenerative disease after Alzheimer's disease.[1]
- Abnormal aggregation of α–Synuclein triggers neuronal death triggering PD.[2]
- Astrocytes are a vital type of glial cells that play a crucial role in supporting brain function and development that propagate intercellular calcium waves for the central nervous system to function.[3]
- Calcium ion signals control fundamental processes for neurons to function such as gene expression, control blood vessel diameter and potassium ion uptake.[4]
- This project studies the effect of different α–Synuclein aggregates on astrocytic calcium waves, a yet unstudied field.

Methodology

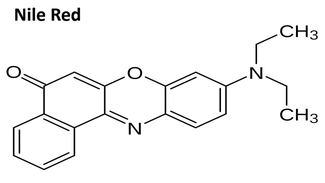
- Lyophilized human recombinant α–Synuclein monomers were used and aggregated at 37 °C at 70 μM in a sodium phosphate buffer.
- Aggregate samples were collected at 3 h, 6 h, 24 h and 96 h after the start of the aggregation.
- Aggregates were characterized using Thioflavin T (ThT) and Nile Red (NR) through Total internal reflection fluorescence (TIRF) imaging (diagram of the molecular structure of NR and THT and the setup of the microscope are to the right).
- α–Synuclein aggregates of 3 h and 96 h were used to treat murine organotypic hippocampal brain slices (OHBS).
- OHBSs were imaged at different magnification (4x, 10x, and 60x) for astrocyte signaling characterization using spinning disc confocal microscopy.
- Astrocytic calcium activity was analyzed using the MATLAB-based platform AQuA (Astrocyte Quantification and Analysis).[5]



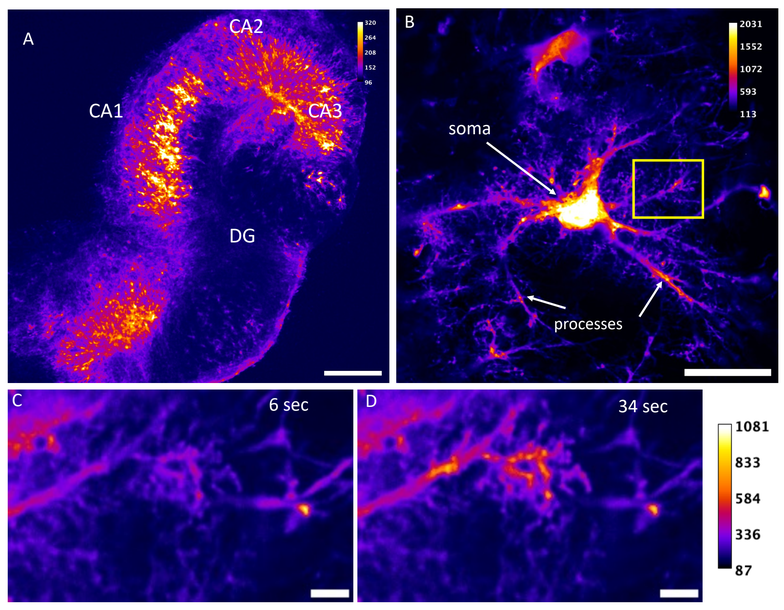
Thioflavin T



Nile Red

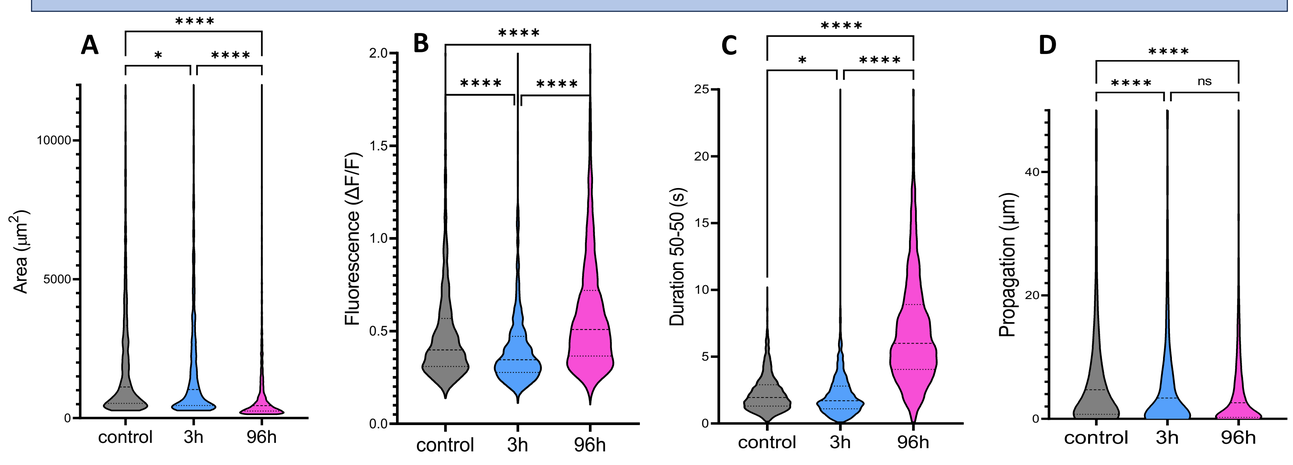


Murine organotypic hippocampal brain slices (OHBS) as *in vitro* model to study astrocyte calcium signaling



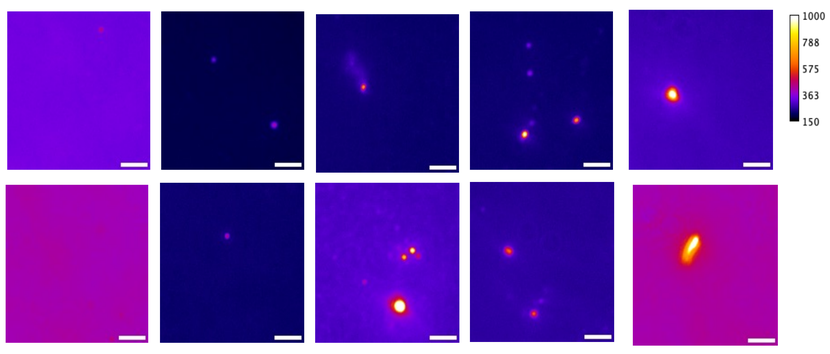
A Representative image of an OHBS with indicated dentate gyrus (DG), and the *cornu ammonis* fields (CA1-CA3). Calcium reporter (GCAMP3) signal is shown in dynamic fire lookup table, calibration bar indicates fluorescence levels (arbitrary units, AU) **B** Maximum intensity projection of GCAMP3 fluorescence signal in a hippocampal astrocyte with soma and processes indicated with white arrows. Scale bar = 500 μm. **C** A magnified image of the highlighted area in **B**, showing an astrocytic process. **D** The astrocytic process with high calcium signaling. Fluorescence can be seen with the calcium reporter (GCAMP3) signal shown in dynamic fire lookup table, calibration bar indicates fluorescence levels (arbitrary units) Scale bar = 50 μm.

Results – astrocytic calcium waves

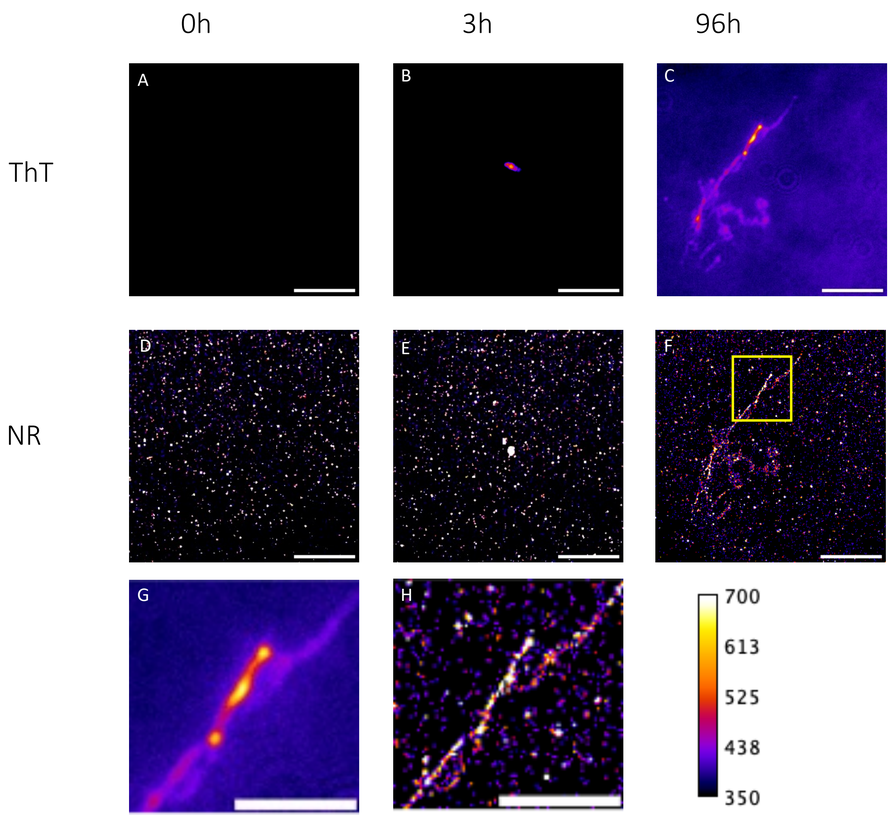


A: α–Synuclein oligomers (3 h) and fibrils (96 h) affected calcium wave area ($H(2) = 804.3$, $p < .0001$ ****), whereby fibrils caused a stronger reduction. **B**: asyn oligomers (3 h) and fibrils (96 h) affected calcium wave area ($H(2) = 618.7$, $p < .0001$ ****), whereby oligomers caused a reduction and fibrils caused an increase. **C**: α–Synuclein oligomers (3 h) and fibrils (96 h) affected calcium wave area ($H(2) = 3351$, $p < .0001$ ****), whereby oligomers caused a marginal decrease in duration and fibrils caused an increase. **D**: α–Synuclein oligomers (3 h) and fibrils (96 h) affected calcium wave area ($H(2) = 56.83$, $p < .0001$ ****), whereby there is no significant difference between oligomers and fibrils. Analysis was conducted using a Kruskal-Wallis test followed by a Dunn post-hoc analysis. 0.1234 (ns), 0.0332 (*), 0.0021 (**), 0.0002 (***), < 0.0001 (****).

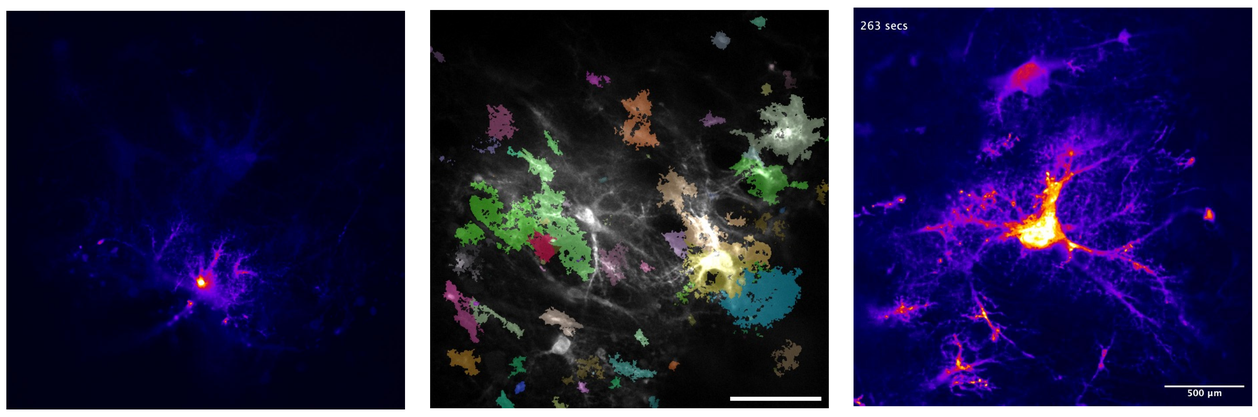
Results – α–Synuclein aggregation and characterization



α-Synuclein aggregates extracted at different times during the aggregation process: Representative images of thioflavin T (ThT) labelled aggregates, obtained using internal reflection microscopy (TIRF), calibration bar indicates fluorescence levels (arbitrary units, AU). Scale bar = 2 μm. (α-Synuclein: 70 μM, kept at 37 °C).



Comparison of Thioflavin T (ThT) and super resolution Nile Red (NR) imaging for α-Synuclein aggregates: **A-C** are representative images of α-Synuclein aggregates stained with ThT, calibration bar indicates fluorescence levels (arbitrary units, AU). **D-F are** corresponding super-resolved images of NR. Scale bar = 5 μm. G-H are magnified images of protein fibril C and F with ThT and NR, respectively. Scale bar = 3 μm.



Future implications

- The research presented here suggests that as fibrils and oligomers have opposite effects on astrocytic calcium waves, there could be two different mechanisms that underlie these changes and have implications for the onset of PD.
- The mechanisms need to be addressed as well as their relevance to the pathology.
- The findings in this project have shown the importance of studying how astrocytes function and how proteins involved in PD such as α–Synuclein influence astrocytic calcium waves.

Conclusions

- The key finding presented in this project is that astrocytic calcium waves are not only affected by the exposure of α–Synuclein, but they are affected in a structure dependent way.
- This shows that fibrils cause long lasting astrocytic calcium waves that are localized and small whereas oligomers cause dimmer astrocytic fibrils and their duration and area marginally change compared to the control astrocytic calcium waves.

Acknowledgements and References

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