

Age-dependent changes of alpha-Synuclein in the motor cortex

Student: Darcy Gabriel

Supervisor: Carola Radulescu

Project location: Department of Brain Sciences, Faculty of Medicine, Imperial College London

Lay Summary:

Alpha-synuclein (aSyn) is a protein found within the nerve cells of the brain. This protein controls the release of nerve signals and interacts with other nerve proteins to regulate neural function (Emamzadeh, 2016). Abnormal accumulations of aSyn lead to the brain disorder Parkinson's Disease (PD) (Van Den Berge et al., 2021). PD, generally associated with older age, causes difficulties with movement, as well as behavioural and functional symptoms (Bloem, Okun and Klein, 2021). Despite its significance, the role of aSyn within the body and ageing process remains unclear. Here we used young (3-months) and older (12-months) mice to investigate how the expression of aSyn and 2 other nerve proteins Synapsin-I (SynI) and HomerI (HI) change with age. We looked at the motor cortex area of the brain, as this has previously shown age-dependent change. We used staining, involving proteins tagged with a light-sensitive marker; and imaging, using a microscope, to visualise these proteins within our samples. We then used two programmes, ImageJ and MATLAB, to quantify protein expression and run statistical tests. We observed an increase in aSyn and a reduction in HI expression in older samples. Subsequently we suggest that aging leads to a change in protein expression within the brain, which may be involved in brain disorders observed in older age.

What was the aim of your project?:

Our study focused on three aims:

1. Optimisation of experimental protocols, imaging and analysis parameters.
2. Identify age-related changes in the size and density peaks in the molecular expression of aSyn, SynI and HI.
3. Determine pre- and postsynaptic colocalisation changes with ageing.

How did you address the aim?

Aim 1 involved antibody optimisation. I tested primary/secondary antibody combinations, concentrations, and incubation times to improve signal detection and reduce nonspecific binding.

Aim 2 focused on identifying age-related changes

in puncta. I used a custom-written MATLAB code and assessed the optimal peak detection parameters through cross-referencing peak detection in specific regions of interest (ROIs) to intensity data from Fiji ImageJ.

Aim 3 focused on determining age-related colocalisation changes in puncta. I verified the overlapping peak detection in the protein fluorescence.

Briefly, the methods involved in this project: adult (P120-390) PC57Bl/6 mice (n=8) were divided into two, sex matched, groups based on age. Brains were sagittally sliced into 40µm-thick sections using a frozen microtome and selected if they contained the motor cortex before primary and secondary antibodies (Figure 1a.) were applied for staining.

Experimental Workflow

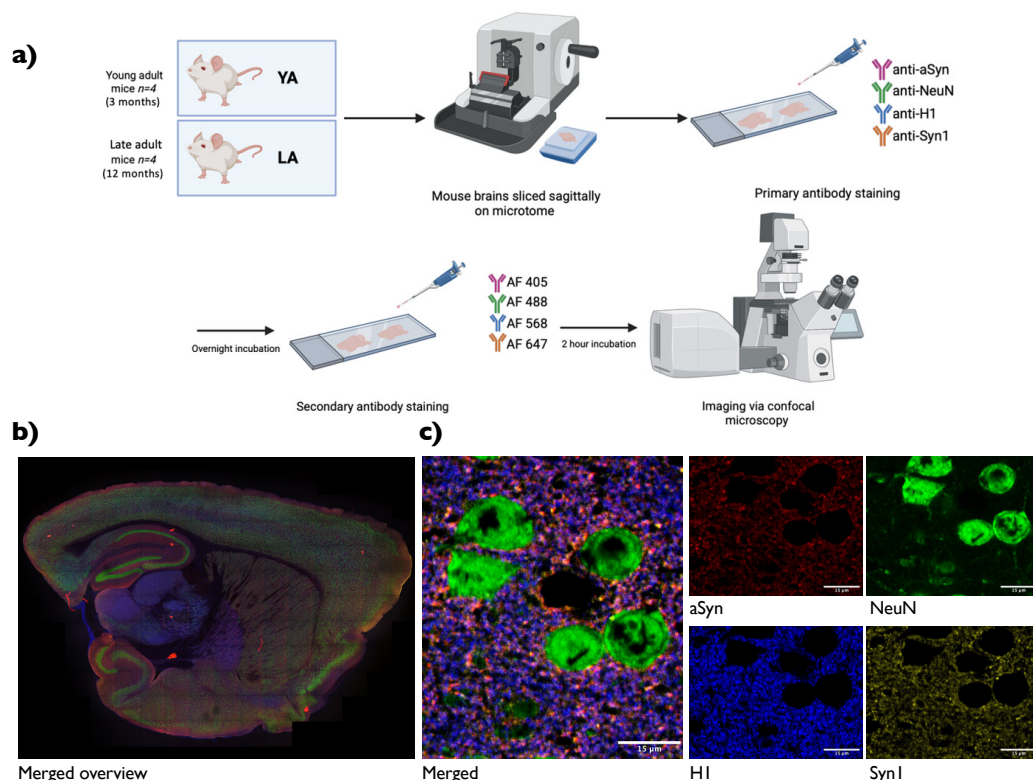


Figure 1. Experimental workflow, representative images and summary of findings. (a) Schematic illustration of experimental workflow. YA = young mice, 3 months old. LA = older mice, 12 months old. Primary antibodies: anti- α -Syn mouse, anti-NeuN guineapig, anti-Homer 1 rabbit, anti-Synapsin I chicken. Secondary antibodies: Goat anti-mouse ALEXA 405 fluorophore (AF 405), goat anti-guineapig ALEXA 488 fluorophore (AF 488), goat anti-rabbit ALEXA 568 fluorophore (AF 568), goat anti-chicken ALEXA 647 fluorophore (AF 647). Created in <https://BioRender.com> (b) Merged overview of a sagittal section showing the motor cortex. (c) Example of high-resolution images from the motor cortex, layer 2/3 expressing aSyn (red), H1 (blue), Syn1 (magenta) or NeuN (green). Merged image of all 4 channels. Scale bar: 15µm.

Imaging used the SP8 Lightning confocal microscope and DM18 platform. Overview images required the 20x/0.4 dry objective lens, 1.00x digital zoom and high-magnification images required 40x/1.30 oil-immersion objective lens, 4.00x digital zoom. Fiji ImageJ and MATLAB were used to analyse molecular expressions. Using Fiji ImageJ, 12 ROIs were selected to measure the EGFP fluorescent signal intensity. A MATLAB code then analysed peaks in synaptic protein expression and colocalisation.

What did you find out?

aSyn is a presynaptic protein involved in regulating neurotransmission and vesicular trafficking (Calabresi et al., 2023). By interacting with other pre- and postsynaptic proteins it facilitates neural function and plasticity (Emamzadeh, 2016). The

abnormal accumulation of aSyn within neurones, referred to as Lewy bodies, is thought to underly the pathophysiology of PD and contribute to disruption of dopaminergic neurones function (Van Den Berge et al., 2021). PD, which is generally associated with older age, causes a syndrome of motor dysfunction and systemic symptoms including gut, sleep and psychiatric disturbance (Bloem, Okun and Klein, 2021). Motor symptoms which include rigidity, bradykinesia and impaired motor learning/adaptation are closely associated with disease pathology within the basal ganglia and motor cortex, making these notable areas for PD and general age-related research (Lindenbach and Bishop, 2013).

At present, the physiological role of aSyn remains poorly understood. Evidence from aSyn knock

out (KO) studies demonstrates a reduction in dopaminergic neurones within the substantia nigra, supporting the assumption that aSyn plays a role in neuronal development. Furthermore, triple KO studies, where all synuclein proteins (alpha, beta, gamma) are removed, demonstrate neurodegenerative impairment (Greten-Harrison et al., 2010). Interestingly, models of aSyn overexpression demonstrated similar deficits within the dopaminergic system. These findings suggest that a coordinated balance of aSyn within the central nervous system is required for physiological function, and that even slight deviations from baseline precipitate significant neurological deficit.

aSyn interacts with multiple presynaptic binding partners, such as the phosphoprotein SynI, to regulate the sequestration of synaptic vesicles within presynaptic boutons (Hoffmann et al., 2023). Imbalances in SynI/aSyn expression potentially precipitate impaired vesicular trafficking hindering neurotransmitter release and overall neural communication (Hoffmann et al., 2021). Although presynaptic mechanisms are central to the function of aSyn, neurotransmission ultimately relies on coordinated pre-/postsynaptic processes (Emamzadeh, 2016). Postsynaptic scaffolds, including

H1, regulate receptor expression and synaptic signalling, and may provide a route through which aSyn may influence synaptic plasticity (Luo et al., 2012, Radulescu et al., 2023). With ageing, the expression and colocalisation of these synaptic proteins may shift, influencing synaptic architecture and plasticity. Overall, changes in protein expression and colocalisation may present a risk factor for neurodegenerative disease, including synucleinopathies such as PD. This work raises the question as to how the molecular expression of pre- and postsynaptic proteins is altered through the ageing process, especially within the higher cortical regions such as the motor cortex.

Here we find that the mean density of aSyn increased from YA to LA motor cortex, mirroring previous work demonstrating an accumulation of aSyn within the aged brain (Saito et al., 2003, Van Den Berge et al., 2021) (Figure 2a). Additionally, we demonstrate a significant reduction in the expression of H1 (Figure 2b), corroborating work showing age-dependent changes within the dendritic spines and reduced capability for synaptic plasticity (Luo et al., 2012). Despite elevation in aSyn expression, we find no significant change in the expression of SynI, suggesting this protein may be less vulnerable to age

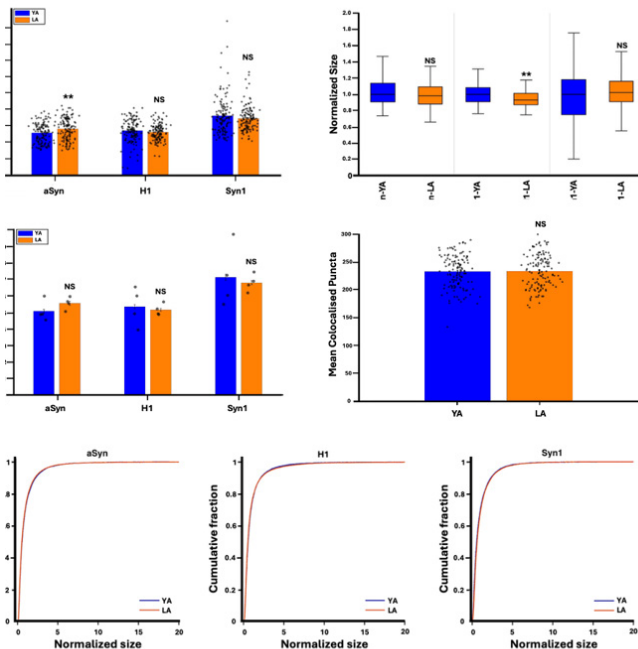


Figure 2. Age-dependent changes in alpha-Synuclein, Homer1, and Synapsin I. Analysis of the motor cortex in YA and LA cohorts. Molecular expression is determined as a ratio between ROI and background signal intensity. Data are presented as mean expression/colocalized puncta ± s.e.m, box plot or cumulative fraction distribution (a) Mean density (per ROI) of aSyn, Homer-1, and SynI. aSyn (YA vs LA, $P=0.0028$), Homer-1 (YA vs LA, $p=0.1920$), SynI (YA vs LA, $P=0.5316$). Two variable t-test (t-test) was used to compare mean density of aSyn and Homer-1. Mann-Whitney U test was used to evaluate molecular expression of SynI. (b) Box plot showing molecular expression of aSyn (YA vs LA, $P=0.1629$), Homer-1 (YA vs LA, $p=0.0016$), SynI (YA vs LA, $P=0.1182$). Mann-Whitney U test was used to evaluate molecular expression of aSyn and Homer-1. t-test was used to evaluate molecular expression of SynI. (c) Mean density (per animal) of aSyn, Homer-1, and SynI. aSyn (YA vs LA, $P=0.2443$), Homer-1 (YA vs LA, $p=0.7625$), SynI (YA vs LA, $P=0.7456$). t-test was used to compare mean density of all proteins. (d) Mean colocalised puncta. Colocalisation of aSyn, Homer-1 and SynI (YA vs LA, $P=0.8854$). t-test was used to compare the extent of colocalisation between cohorts. (e) Peak size plotted as a cumulative fraction distribution. aSyn (YA vs LA, $P=5.7921e-04$). Homer-1 (YA vs LA, $P=0.0153$). SynI (YA vs LA, $P<1.5751e-23$). * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$, NS not significant.

related change (Figure 2a, Figure 2c).

The co-expression of pre- and postsynaptic proteins was shown to be associated with the regulation of neurotransmission and neural plasticity (Radulescu et al., 2023). When evaluating the mean colocalised puncta in YA vs LA samples, we find no change in the number of colocalised puncta (Figure 2d). Our findings suggest that interactions between the pre- and postsynaptic remain stable through ageing to maintain their connection and neurotransmission.

With previous work in mind, we conclude that multi-protein expression changes occur in the ageing brain and may be involved in the pathophysiology of age related proteinopathies such as PD. Future work in the lab will use expansion microscopy and cyclic immunofluorescence to investigate the colocalisation of a greater number of proteins that may contribute to age-dependent mechanisms of PD. Specifically, we will use an animal model of synucleinopathies to understand age-related changes in aSyn aggregation and presynaptic protein expression. Through this work we will investigate synaptic dysfunction that may contribute to overall neurological decline.

What did you learn from participating in this project?

Participating in this project provided the opportunity to gain hands-on experience across various aspects of laboratory work such as brain sectioning, immunofluorescent staining, confocal imaging, data analysis and writing-up of reports. I particularly enjoyed the challenge of learning to use the confocal microscope. In a previous project with Barnes Lab, I worked on analysing pre-acquired images. However, this was my first opportunity to acquire the images myself. This experience felt like a natural progression from my earlier work, building upon my previous understanding. Additionally, I was able to deepen my understanding of data analyses, using Fiji ImageJ in combination with statistical software such as MATLAB to interpret our experimental data.

Other skills that I learnt through this project include free-floating immunofluorescent staining, cryostat sectioning, and basic coding in MATLAB.

Overall, this experience has furthered my understanding of how microscopy can be used to understand cell and synapse physiology and the pathological mechanisms underlying neurodegenerative disease. It has affirmed my interest in pre-clinical research and taught me a range of valuable skills that I will take forward into my academic medical career.

How has this project affected your long-term goals?

This project has provided a valuable opportunity to explore microscopy and its applications in pre-clinical research. The experience has deepened my interest in research, particularly in the area of neurodegenerative disease.

My time on this project has highlighted how much I enjoy all aspects of the research process, from reading and understanding the existing literature to producing and analysing original data.

As a result of both my BSc in Neuroscience and Mental Health, and my recent experience on this project, I am now eager to pursue a PhD in neuroscience soon after completing my medical studies. Not only will this prove invaluable for my medical career, but it will allow me to engage in research alongside clinical practice. In the short term, I hope to present the findings from this project at the RMS Beginners' Competition. This will provide an opportunity to share our work and further develop my presentation skills, an essential skill for the career I hope to build.

Reference list

- Bloem, B.R., Okun, M.S. and Klein, C. (2021). Parkinson's Disease. *The Lancet*, [online] 397(10291), pp.2284–2303. doi:[https://doi.org/10.1016/S0140-6736\(21\)00218-X](https://doi.org/10.1016/S0140-6736(21)00218-X).
- Calabresi, P., Mechelli, A., Natale, G., Volpicelli-Daley, L., Di Lazzaro, G. and Ghiglieri, V. (2023). Alpha-synuclein in Parkinson's disease and other synucleinopathies: from overt neurodegeneration back to early synaptic dysfunction. *Cell Death & Disease*, [online] 14(3), pp.1–16. doi:<https://doi.org/10.1038/s41419-023-05672-9>.
9. Emamzadeh, F. (2016). Alpha-synuclein structure,



The Barnes Laboratory team.



Darcy Gabriel.

functions, and interactions. *Journal of Research in Medical Sciences*, [online] 21(1), p.29. doi:<https://doi.org/10.4103/1735-1995.181989>.

Greten-Harrison, B., Polydoro, M., Morimoto-Tomita, M., Diao, L., Williams, A.M., Nie, E.H., Makani, S., Tian, N., Castillo, P.E., Buchman, V.L. and Chandra, S.S. (2010). $\alpha\beta\gamma$ -Synuclein triple knockout mice reveal age-dependent neuronal dysfunction. *Proceedings of the National Academy of Sciences*, 107(45), pp.19573–19578. doi:<https://doi.org/10.1073/pnas.1005005107>.

Hoffmann, C., Rentsch, J., Tsunoyama, T.A., Chhabra, A., Aguilar Perez, G., Chowdhury, R., Trnka, F., Korobeinikov, A.A., Shaib, A.H., Ganzella, M., Giannone, G., Rizzoli, S.O., Kusumi, A., Ewers, H. and Milovanovic, D. (2023). Synapsin condensation controls synaptic vesicle sequestering and dynamics. *Nature Communications*, [online] 14(1), p.6730. doi:<https://doi.org/10.1038/s41467-023-42372-6>.

Hoffmann, C., Sansevrino, R., Morabito, G., Logan, C., Vabulas, R.M., Ulusoy, A., Ganzella, M. and Milovanovic, D. (2021). Synapsin Condensates Recruit alpha-Synuclein. *Journal of Molecular Biology*, 433(12), p.166961. doi:<https://doi.org/10.1016/j.jmb.2021.166961>.

Lindenbach, D. and Bishop, C. (2013). Critical Involvement of the Motor Cortex in the Pathophysiology and Treatment of Parkinson's Disease. *Neuroscience and biobehavioral reviews*,

[online] 37(10 0 2). doi:<https://doi.org/10.1016/j.neubiorev.2013.09.008>.

Luo, P., Li, X., Fei, Z. and Poon, W. (2012). Scaffold protein Homer 1: Implications for neurological diseases. *Neurochemistry International*, 61(5), pp.731–738. doi:<https://doi.org/10.1016/j.neuint.2012.06.014>.

Radulescu, C.I., Nazanin Doostdar, Nawal Zabouri, Leire Melgosa-Ecenarro, Wang, X., Sadra Sadeh, Pavlina Pavlidi, Airey, J., Kopanitsa, M.V., Clopath, C. and Barnes, S.J. (2023). Age-related dysregulation of homeostatic control in neuronal microcircuits. *Nature Neuroscience*. doi:<https://doi.org/10.1038/s41593-023-01451-z>.

Saito, Y., Kawashima, A., Ruberu, N.N., Fujiwara, H., Koyama, S., Sawabe, M., Arai, T., Nagura, H., Yamanouchi, H., Hasegawa, M., Iwatsubo, T. and Murayama, S. (2003). Accumulation of Phosphorylated α -Synuclein in Aging Human Brain. *Journal of Neuropathology & Experimental Neurology*, 62(6), pp.644–654. doi:<https://doi.org/10.1093/jnen/62.6.644>.

Van Den Berge, N., Ferreira, N., Mikkelsen, T.W., Alstrup, A.K.O., Tamgüney, G., Karlsson, P., Terkelsen, A.J., Nyengaard, J.R., Jensen, P.H. and Borghammer, P. (2021). Ageing promotes pathological alpha-synuclein propagation and autonomic dysfunction in wild-type rats. *Brain*, 144(6), pp.1853–1868. doi:<https://doi.org/10.1093/brain/awab061>.