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Impact of alpha-synuclein pathology on adult neurogenesis: evidence for multilayered mechanisms

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Parkinson's disease (PD) is the second most common neurodegenerative disease affecting 8–10 million people worldwide. Moreover, PD is the fastest-growing neurodegenerative disease and it is predicted that the number of patients will double in the next thirty years. Neuropathologically, PD is characterized by the presence of protein aggregations called Lewy bodies (LB) and by degeneration of dopaminergic neurons in the substantia nigra pars compacta, which give rise to classical motor symptoms. However, overwhelming scientific evidence shows that PD is a multilayered disease. LB pathology affects different brain regions and neuronal populations leading to various non-motor symptoms, such as cognitive, sleep, and sensory impairments, mood and metabolic disorders, etc., some of which, interestingly, appear long before the hallmark motor symptoms. A main component of LB is the protein alpha-synuclein (α-syn). Accumulation and aggregation of α-syn is a characteristic feature that can be observed in synucleinopathies, a group of diseases which PD, dementia with Lewy bodies (DLB), and multiple system atrophy belongs to. While DLB is characterized by progressive dementia, about 80% of PD patients experience some form of cognitive impairment. Therefore, PD and DLB together constitute the second leading cause of neurodegenerative dementias. Moreover, it is proposed that α-syn-associated pathology in cortical and hippocampal (Hipp) brain areas is causing cognitive deficits and dementia in PD and DLB. Alterations in adult neurogenesis may represent a potential pathomechanism.

Adult neurogenesis: locations, mechanisms, and functional implications: Altman and Das were the first to introduce the idea that neurogenesis is not restricted to embryonic, perinatal, and early postnatal stages in mammals but that it occurs in adult individuals as well (Abdissa et al., 2020). Brain regions canonically considered capable to provide an environment for adult neural progenitor cell (NPCs) maintenance are the subgranular zone (SGZ), part of the granular cellular layer of the dentate gyrus (DG) of the Hipp, and the subventricular zone, surrounding the lateral ventricles. In recent years more non-canonical regions were described and include brain stem, cerebellum, corpus callosum, Hipp (Ammon's horn), hypothalamus, neocortex, olfactory tubercle, piriform cortex, septum, striatum, substantia nigra, and thalamus. The SGZ provides a suitable niche in which NPCs proliferate, migrate, and differentiate in DG granule neurons. Several NPCs developmental stages are identified and described. The earliest NPCs are radial glial-like cells or type 1 cells. These cells represent the sub-population of the NPCs that are capable of generating intermediate neuronal progenitor cells (iNPCs), type 2 cells, as well as glial, astrocyte cells. iNPCs have short-lasting amplifying characteristics and produce neuroblasts type 3 cells, which differentiate first into immature neurons and finally into mature DG neurons. This final step, the transition from immature to mature neurons in adults strongly resembles the neuronal maturation at the embryonic stage. The neurovascular unit and astrocytes provide the environment and trophic factors required by differentiating neurons (Gonçalves et al., 2016). At the early stage of maturation, neurons are characterized by an irregular shape, immature neuronal spikes, and a lack of synaptic activity. While still exhibiting spineless dendrites, neurons begin to receive slow GABAergic input. Shortly after, glutamatergic afferents are observable in neurons displaying mature excitability and morphology. At the final step of neuronal maturation, fast GABAergic inputs are established. Therefore, the timing for neuronal maturation in the adult Hipp is modulated by local network activity, and it closely mimics hippocampal embryonic development (Gonçalves et al., 2016).

Stage-specific morphological features, as well as signaling mechanisms that drive the process of adult neurogenesis and neuronal differentiation have been characterized (**Figure 1**). The time-dependent manner of changes in NPC morphology is accompanied by genetically distinguished stages in which expression of specific mRNA and protein markers can be observed (Gonçalves et al., 2016). To comprehend the proportion of adult neurogenesis and therefore its importance, it is estimated that in the adult rodent brains several thousand new neurons are born every day. Since the original studies from Altman and Das (Abdissa et al., 2020), it was shown that adult neurogenesis processes are involved in maintaining brain tissue homeostasis and play a major role in both health and disease.

In SGZ, processes of adult neurogenesis culminate with the functional integration of young granule neurons in the molecular layer of the DG. Adult-born neurons significantly contribute to Hipp dependent functions, such as memory encoding and mood regulation. Moreover, impairments in adult neurogenesis have been associated with many brain function disorders and diseases including PD and DLB. Therefore, impaired adult neurogenesis is potentially involved in Hipp functional decline observable as cognitive deficits and dementia in PD and DLB.

Evidence for impairments of adult neurogenesis in patients with PD and DLB: Utilizing samples obtained post mortem from PD and DLB patients to investigate adult neurogenesis processes is technically difficult and therefore limited. Several authors aimed to elucidate the role of dopamine and dopamine loss in PD on adult neurogenesis and observed conflicting results (Lim et al., 2018). While it was established that NPCs express the dopamine receptor and receive dopaminergic afferents, consensus about the extent of adult neurogenesis impairment in PD and how dopamine affects it, could not be reached. In fact, while α-syn may impair adult neurogenesis in PD indirectly through toxic misfolding in dopaminergic neurons, an additional direct gain of toxic function in the Hipp is highly probable. In support of this hypothesis, α-syn in its physiological form alters processes of adult neurogenesis (Winner et al., 2012), suggesting that augmentation in protein levels alone could be a culprit. Thus, Lewy body pathology and neurodegeneration reported in PD and DLB patients in the Hipp, is likely to impact adult neurogenesis processes. This highlights the complexity of adult neurogenesis processes and a need for further in-depth studies into potential pathomechanisms.

Lessons from animal models of synucleinopathies: Animal models are obviously indispensable to studying the effect of α-syn pathology on adult neurogenesis. Winner et al. (2012) showed that α-syn levels modulate adult neurogenesis, and the development of dendritic arborization and spines in the DG using transgenic mice overexpressing human WT α-syn versus α/β-syn knockout mice. In DLB patients, they observed a decrease of SOX2 (a marker of early NPCs) positive cells and reductions in dendrite numbers, dendrite length and branching, and spine density in striatal medium spiny neurons. Altogether, the obtained data suggested α-syn as a direct negative modulator of adult neurogenesis processes (Winner et al., 2012). This is confirmed in studies on mice expressing

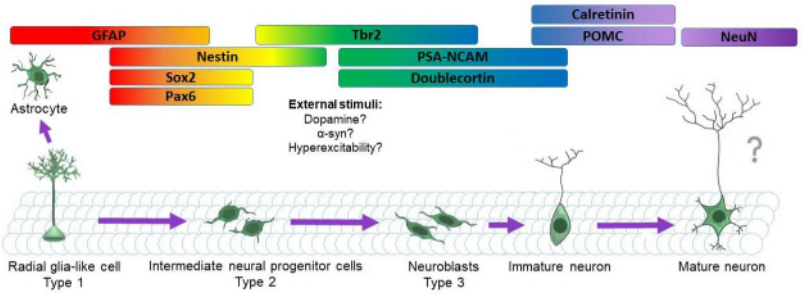

Figure 1 | Developmental processes of adult neurogenesis in the subgranular zone (SGZ) of the hippocampus (Hipp).
Neuronal progenitor cells (NPCs) in the Hipp: radial glia-like cells, type 1 cells; intermediate neuronal progenitor cells (iNPCs), type 2 cells; neuroblasts (NBs), type 3 cells; immature neurons; mature neurons and their differentiation. Some of the stage-specific NPCs markers: early NPCs markers (type 1/2 cells): glial fibrillary acidic protein (GFAP), nestin, paired box 6 (Pax6), sex determining region Y-box 2 (Sox2); late NPCs markers (type 2/3 cells): doublecortin, polysialylated-neural cell adhesion molecule (PSA-NCAM), T-box brain protein 2 (Tbr2); immature neuronal markers: calretinin, pro-opiomelanocortin (POMC); pan neuronal marker: hexaribonucleotide binding protein-3 (NeuN).

human WT or mutated A53T α-syn under the platelet-derived growth factor-β promoter, which observed a reduction of the number of proliferating cell nuclear antigen (marker of cell proliferation) positive cells in both the SGZ and subventricular zone. In the SGZ, this was accompanied by decreased Notch-1, NICD, and Hes-5 expression suggesting that α-syn impairs the survival of NPCs by interfering with the Notch signaling pathway (Crews et al., 2008). Moreover, in A53T mice it was observed that chronic fluoxetine treatment ameliorates adult neurogenesis impairment by increasing brain-derived neurotropic factor and glial cell-derived neurotrophic factor expression (Micheli et al., 2018). Another study conducted on conditional tet-off transgenic mice overexpressing α-syn, a change in proliferating cell nuclear antigen positive cell numbers was not detected. However, a reduction in BrdU (number of newly generated neurons) and doublecortin (late NPCs marker), positive cell numbers were observed (Nuber et al., 2008). Mice overexpressing rare mutated forms of α-syn are widely used, but the vast majority of patients do not harbor mutations in the protein. Instead, it is the pathological accumulation of the WT protein which constitutes LB pathology and potential toxic mechanisms. Mice that overexpress human WT alpha-synuclein under the control of the murine Thy-1 promoter (Thy1-aSyn, line 61) (Chesselet et al., 2012) replicate α-syn pathology observed in PD and DLB patients as well as characteristic motor and of non-motor symptoms, including early cognitive deficits. Interestingly, our first insights into neurogenesis processes in the DG of these mice (under review in Frontiers Cell and Developmental Biology), suggest proliferating cells/NPCs population in SGZ. Nevertheless, it should be mentioned that in an earlier study observing adult neurogenesis in Thy1-aSyn mice did not detect statistically significant differences in proliferating cell nuclear antigen positive cell numbers (Regensburger et al., 2018). Aside from different approaches used to quantify the number of NPCs in Regensburger et al. and our study, different observations could be explained by the age of the animals used in the experiment. While in a study by Regensburger et al. (2018), 4 month-old animals were used, we utilized 6–7-month-old mice suggesting that gross adult neurogenesis impairment starts at a later time point. Contrary to other transgenic mice overexpressing α-syn used in aforementioned studies, at an early stage of the disease progression, prior to dopamine loss, our data suggest that Thy1-aSyn mice seem to present with an increase of proliferating cells in the DG. Further studies are warranted to understand how this impacts adult neurogenesis processes and the number of mature neurons in the DG.

Potential mechanisms of how α-syn could aberrantly affect adult neurogenesis: Firstly, it was established that dopamine neurons project from the substantia nigra pars compacta to the SGZ, that granule neurons as well as NPCs possess dopamine receptors, and that dopamine plays an important role in NPCs proliferation, migration, and differentiation, while dopamine depletion in mice and *in vitro* negatively affects adult neurogenesis (Shohayeb et al., 2018). An increase in extracellular dopamine levels was observed in young Thy1-aSyn mice (Chesselet et al., 2012). Whether this contributes to alterations in the processes of adult neurogenesis in Thy1-aSyn mice represents an intriguing question. Secondly, α-syn may affect mechanisms of cell cycle regulation. An *in vitro* study utilizing an SH-SY5Y cell line transfected with human WT α-syn showed that α-syn induces cytotoxicity and at the same time, promotes cell proliferation. Moreover, α-syn increased expression of senescence and mitosis markers in SH-SY5Y cell line. In another study conducted in PC12 cells engineered to conditionally induce α-syn expression, α-syn increased the proliferation rate and number of cells in the S phase of the cell cycle. Upregulation of mitosis markers and downregulation of the tumor suppressor markers was observed as well. In a recent study, Findeiss et al. (2021) proposed the role of miRNA in the control of the expression of cell cycle genes. Authors used Lund human mesencephalic cell line, and performed a comprehensive miRNome-wide screen. They observed differential expression of different miRNAs while target gene mining analysis showed an enrichment of the cell cycle signaling pathways, more precisely the significant differential expression levels of six cell cycle genes in SNCA-overexpressing post-mitotic neurons was detected. In short, this study described that early-stage intracellular accumulation of α-syn in human mesencephalic post-mitotic neurons is accompanied by an altered expression of miRNAs, leading to an enrichment of cell cycle genes (Findeiss et al., 2021). In contrast to what *in vitro* studies suggest, *in vivo* experiments propose that the absence of α-syn promotes adult neurogenesis processes. Considering the fact that Thy1-aSyn mice overexpress human WT α-syn, unphysiological presence of misfolded protein, and/or loss of function of murine protein could potentially impact adult neurogenesis. Thirdly, network hyperexcitability and seizures can be observed in some Alzheimer's disease, frontotemporal dementia, and DLB patients. Interestingly, Thy1-aSyn mice show Hipp circuitry remodeling and network hyperexcitability. Moreover, aberrant network excitability that can escalate into seizure activity was observed in Thy1-aSyn and A53T mice while nearly one-quarter of DLB patients showed cortical myoclonus (Peters et al., 2020). It is well documented that epileptic seizures promote adult neurogenesis in both humans and animal models of the disease. Therefore, the presence of network hyperexcitability could alter neurogenesis processes in Thy1-aSyn mice and in PD/DLB especially at early disease stages. Morris et al. observed a reduction of calbindin expression in the granular cellular layer of Thy1-aSyn mice, which could indicate lower survival of these neurons in Thy1-aSyn mice caused by α-syn burden. An increase in neurogenesis could represent a compensatory response, however, further studies need to clarify whether these neurons differentiate and integrate sufficiently for a functional benefit.

Therapeutic implications of adult neurogenesis processes in PD and DLB: While cognitive decline is a common symptom in PD and appears early in a significant fraction of patients, DLB is characterized by dementia. In both diseases, there are few treatment options with limited effects on cognitive loss and dementia. As neural stem cells are a potential source for endogenous repair, modulating the processes of adult neurogenesis is a promising strategy for intervention in neurodegenerative diseases. Deciphering the processes of altered adult neurogenesis in these diseases can potentially lead to more effective and specific therapeutic approaches.

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