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# The role of glial cells in alpha- synuclein pathology

*Focus on degradation, cell-to-cell propagation and  
inflammation*

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### **Abstract**

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Growing evidence emphasizes the role of astrocytes and microglia in Parkinson's disease (PD) and Alzheimer's disease (AD). Yet, little is known about their impact on specific disease processes and if their involvement is beneficial or detrimental. The aim of this thesis was to further investigate the role of astrocytes and microglia in PD and AD. To this purpose, cultured human astrocytes and microglia were exposed to aggregates of alpha-synuclein ( $\alpha$ SYN) or amyloid-beta ( $A\beta$ ), proteins that are central to PD and AD brain pathology, respectively.

In Paper I, the toxicity and cell-to-cell spreading of aggregated  $\alpha$ SYN in human astrocytes were evaluated. We found that astrocytes can engulf large amounts of  $\alpha$ SYN aggregates, which are stored inside the cells instead of being degraded. This intracellular storage was found to result in severe cellular stress. As a response, stressed astrocytes were shown to transfer  $\alpha$ SYN via tunneling nanotubes (TNT) to healthy astrocytes.

T cells have been observed to enter the PD brain, but little is known about which stationary cell types they interact with. In Paper II, the ability of astrocytes and microglia to act as antigen presenting cells in the presence of aggregated  $\alpha$ SYN was investigated. Both astrocytes and microglia were capable of expressing major histocompatibility class I (MHC I) and MHC II. However, only astrocytes had the capacity to express other molecules crucial for T-cell activation, such as CD80 and CD86. MHC II expressing astrocytes were also found in close vicinity to T cells in the PD brain.

In paper III, the cross-talk between microglia and astrocytes in the presence of  $\alpha$ SYN and  $A\beta$  aggregates was examined. When cultured separately, microglia appeared to degrade  $\alpha$ SYN and  $A\beta$  better than astrocytes. However, co-culture experiments showed that microglia and astrocytes have a synergistic effect on the clearance of protein aggregates. Cell-to-cell contact was revealed as one of the possible mechanisms by which astrocytes and microglia communicate with each other.

In Paper IV, the molecular mechanisms by which the compound KYP-2407 enhances  $\alpha$ SYN clearance was investigated. We found that KYP-2407 stimulates the auto-lysosomal pathway in the presence of  $\alpha$ SYN aggregates. Calpain proteins, which increase  $\alpha$ SYN aggregation and diminish autophagy in PD, were also shown to be reduced in the presence of KYP-2407.

Taken together, this thesis contributes with novel and important knowledge to the potential role of astrocytes and microglia in PD and AD.

*Keywords:* Parkinson's disease, alpha-synuclein, amyloid-beta, astrocytes, microglia, tunneling nanotubes, antigen presentation, T cells, glial crosstalk, degradation, KYP-2407

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*Till min älskade familj för allt ni  
gjort och gör för mig*



*If you take away flowers from my poems  
One of my four seasons will die  
If you take away love,  
Two seasons will die  
If you take away bread,  
Three seasons will die  
If you take away freedom,  
My whole year will die and so will I*

*-Sherko Bekas*

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# List of Papers

This thesis is based on the following papers, which are referred to in the text by their Roman numerals.

- I **Rostami J**, Holmqvist S, Lindström V, Sigvardson J, Westermarck GT, Ingelsson M, Bergström J, Roybon L and Erlandsson A. (2017) Human astrocytes transfer aggregated alpha-synuclein via tunneling nanotubes. *Journal of Neuroscience*, 37(49):11835-11853.
- II **Rostami J**, Fotaki G, Russ K, Bergström J, Essand M, Healy LM and Erlandsson A. (2020) Astrocytes have the capacity to act as antigen-presenting cells in the Parkinson's disease brain. *Journal of Neuroinflammation*, 17(1):119.
- III **Rostami J**, Mothes T, Kolahdouzan M, Eriksson O, Moselem M, Bergström J, Ingelsson M, O'Callaghan P, Healy L, Falk A, Erlandsson A. Cross-talk between astrocytes and microglia results in increased degradation of  $\alpha$ -synuclein and amyloid- $\beta$  aggregates. *Manuscript*.
- IV **Rostami J**, Jäntti M, Cui H, Rinne M.K, Kukkonen J.P, Falk A, Erlandsson A, Myöhänen T. (2020) Prolyl oligopeptidase inhibition by KYP-2407 increases alpha-synuclein fibril degradation in neuron-like cells. *Biomedicine and Pharmacotherapy*, 131:110788.

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## Additional papers

- V Ben-David Y, Kagan S, Cohen Ben-Ami H, **Rostami J**, Mizrahi T, Thakur G, Vaknin-Dembinsky A, Healy LM, Brenner T and Treinin M. (2020) RIC3, the cholinergic anti-inflammatory pathway, and neuroinflammation. *Journal of International Immunopharmacology*, 83:106381.
- VI Gustafson G, Lindström V, **Rostami J**, Nordström E, Lannfelt L, Bergström J, Ingelsson M, Erlandsson A. (2017) Alpha-synuclein oligomer-selective antibodies reduce intracellular accumulation and mitochondrial impairment in alpha-synuclein exposed astrocytes. *Journal of Neuroinflammation*, 14(1):241.
- VII Lekholm E, Perland E, Eriksson MM, Hellsten SV, Lindberg FA, **Rostami J** and Fredriksson R. (2017) Putative Membrane-Bound Transporters MFSD14A and MFSD14B Are Neuronal and Affected by Nutrient Availability. *Frontiers in Molecular Neuroscience*, 10:11.

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# Abbreviations

aa	Amino acids
A $\beta$	Amyloid-beta
A $\beta$ -F	Amyloid-beta fibrils
AD	Alzheimer's disease
ALP	Autophagy-lysosomal pathway
APC	Antigen-presenting cell
APP	Amyloid precursor protein
$\alpha$ SYN	Alpha-synuclein
$\alpha$ SYN-F	Alpha-synuclein fibrils
ATP	Adenosine triphosphate
BBB	Blood-brain barrier
bFGF	Basic fibroblast growth factor
CMA	Chaperone-mediated autophagy
CNTF	Ciliary neurotrophic factor
COMT	Catechol-o-methyl transferase
CSF	Cerebrospinal fluid
DAPI	4', 6-diamidino-2-phenylindol
DLB	Dementia with Lewy bodies
EE	Early endosomes
ELISA	Enzyme-linked immunosorbent assay
ER	Endoplasmic reticulum
ESC	Embryonic stem cell
FBS	Fetal bovine serum
GFAP	Glial fibrillary acidic protein
Hsp-70	Heat shock protein 70
HNE	4-hydroxy-nonenal
HPC	Hematopoietic progenitor cell
HRP	Horseradish peroxidase
ICC	Immunocytochemistry
IGF-1	Insulin-like growth factor 1
IHC	Immunohistochemistry
iPSC	Induced pluripotent stem cell
LAMP	Lysosomal-associated protein
LB	Lewy body
LC3	Microtubule associated protein 1 light-chain 3
LE	Late endosomes

LN	Lewy neurite
MCSF	Macrophage colony-stimulating factor
MHC	Major histocompatibility complex
MMSE	Mini-mental state examination
MRI	Magnetic resonance imaging
MSA	Multiple system atrophy
MTS	Mitochondrial transfer sequence
NES	Neuroepithelial-like stem cells
PD	Parkinson's disease
PET	Positron emission tomography
PREP	Prolyl oligopeptidase
PRR	Pattern recognition receptor
PSEN	Presenilin
PTM	Post-translational modifications
PVDF	Polyvinylidene fluoride
ROS	Reactive-oxygen species
SNARE	Soluble N-ethylmaleimide-sensitive factor attachment protein receptor
SNC	Substantia nigra pars compacta
TEM	Transmission electron microscopy
TLR	Toll-like receptor
TNT	Tunneling nanotube
TSA	Tyramide signal amplification
UPP	Ubiquitin-proteasome pathway
WB	Western blot

# Introduction

This thesis focuses mainly on alpha-synuclein ( $\alpha$ SYN) pathology in Parkinson's disease (PD). However, amyloid-beta ( $A\beta$ ) aggregates, which are of importance for Alzheimer's disease (AD), were also studied in Paper III. Hence, I describe both diseases in the background, but put most emphasis on  $\alpha$ SYN pathology and PD.

## Neurodegenerative disorders

Neurodegenerative disorders is an umbrella term for various conditions such as amyloidosis,  $\alpha$ -synucleinopathies and TDP-43 proteinopathies, causing neuronal cell death in the central or peripheral nervous system [1]. Although characterized by different protein aggregates and anatomic vulnerability, neurodegenerative disorders share many central pathological processes that are involved in the progression of each disease. These include: protein abnormalities and aggregation, oxidative stress, dysfunctional degradation and neuroinflammation [1]. The most frequent neurodegenerative disorders are AD with a prevalence of 30-40 million people worldwide and PD, which affects about 6 million people in the world [2,3]. The vast majority of AD and PD cases (> 90%) are sporadic where the age of onset is above 60, making age the biggest risk factor for both diseases. The number of AD and PD cases have doubled since the 1990s and are expected to continue rising due to the increasing average life-span [2,3]. The diagnoses of these diseases are based on the medical history of the patient, symptom description, mini-mental state examination (MMSE), analysis of the cerebrospinal fluid (CSF) and imaging of the brain using magnetic resonance imaging (MRI) or positron emission tomography (PET). However, the diagnostic gold standard for neurodegenerative disorders is *post mortem* neurological evaluation as many patients exhibit mixed clinical features. This is in part explained by AD and PD patients sharing many symptoms with other neurodegenerative disorders. Hence, disease specific biomarkers are crucial to evaluate novel treatments. Presently, treatments available for AD and PD only relieve the symptoms. There is thus no cure and no therapy that affects the progression of the diseases.

## Parkinson's disease

Histopathological characterization of PD includes presence of intraneuronal Lewy bodies (LB) and Lewy neurites (LN) that consist mainly of fibrillary  $\alpha$ SYN ( $\alpha$ SYN-F) [4,5] (Figure 1). Chronic neuroinflammation, including gliosis and infiltration of peripheral immune cells to the brain, is another characteristic of the PD brain [6]. In addition to neurons,  $\alpha$ SYN inclusions appear frequently in astrocytes in the PD brain at all stages of the disease [7–9]. Intracellular  $\alpha$ SYN-F are also found in dementia with Lewy body (DLB) and in multiple system atrophy (MSA). Together, these three diseases are classified as  $\alpha$ -synucleinopathies [10].

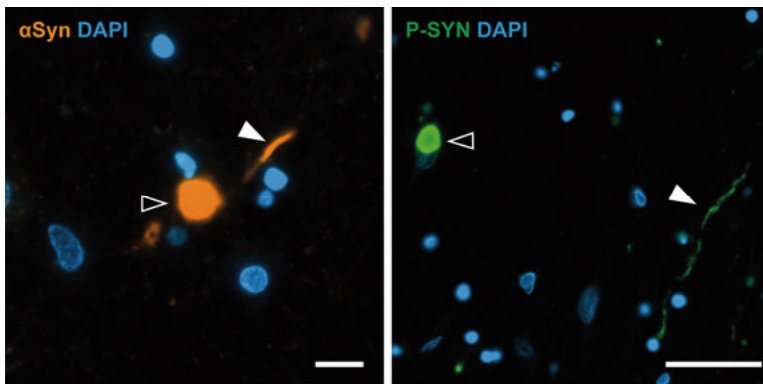


Figure 1. Presence of a LBs (unfilled arrows) and LNs (filled arrows) in a *post mortem* PD brain stained with anti- $\alpha$ SYN antibody (orange) and anti-pser129  $\alpha$ SYN antibody (green). Scale bars = 10  $\mu$ m.

Patients with PD exhibit four typical motor symptoms: bradykinesia or slowness of movement, resting tremor, rigidity and impaired posture balance. These symptoms mainly arise due to loss of dopaminergic neurons in the substantia nigra pars compacta (SNc) giving rise to reduced dopamine levels [11]. Non-motor related symptoms due to neuronal loss in other brain regions during PD include loss of smell, sleep disturbances, depression and cognitive impairment [12]. Parkinson's disease arises in both sporadic and genetic forms. Although the vast majority of cases are sporadic, up to 10% of cases are due to hereditary factors [13,14]. Duplications and triplications of the  $\alpha$ SYN encoding gene, *SNCA*, as well as missense mutations in *SNCA* have been shown to cause familial cases of  $\alpha$ -synucleinopathies [15–19]. Moreover, mutations in *PINK-1*, *LRRK2*, *Parkin* and *DJ-1* specifically cause familial PD [20–24]. Interestingly, all of the above-mentioned genes are involved in functions related to mitochondria, autophagy and lysosomal degradation.

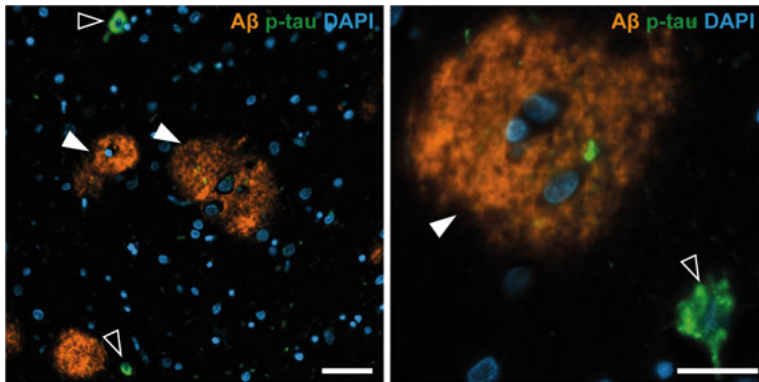
## Alpha-synuclein

Alpha-synuclein is a small protein of 140 amino acids (aa) (14 kDa) that is expressed in almost all tissues of the body, but most abundantly in the brain [25,26]. The protein sequence is divided into three distinct regions: a positively charged N-terminus (aa 1-60), a hydrophobic mid-region (aa 61-95) and an acidic C-terminus (aa 96-140). The N-terminus part of  $\alpha$ SYN can adopt an  $\alpha$ -helical structure and interacts with lipids [27]. This is possible due to the presence of seven series of 11-aa repeats that includes a KTKEGV motif, resulting in a lysine-rich region [28]. The mid-region of  $\alpha$ SYN was first discovered in A $\beta$  plaques extracted from AD brains, which led to the discovery of the  $\alpha$ SYN protein [29]. The C-terminus part of  $\alpha$ SYN is negatively charged, hydrophilic, and binds to metals and other proteins. Furthermore, this region is homologous to heat-shock proteins, suggesting that  $\alpha$ SYN has a chaperone function [30–32]. The exact functions of  $\alpha$ SYN remain unknown. However, due to its cellular localization in the synapses,  $\alpha$ SYN is believed to be important for synaptic functions, such as neurotransmitter release. For example,  $\alpha$ SYN has been suggested to act as a molecular chaperone for soluble N-ethylmaleimide-sensitive factor attachment protein receptor (SNARE) proteins involved in the vesicle budding and release of neurotransmitters from the pre-synaptic cleft [33,34]. Interestingly,  $\alpha$ SYN also has a mitochondrial targeting sequence (MTS), which transports nuclear-encoded mitochondrial proteins to mitochondria by interacting with receptors on the mitochondrial outer membrane [35]. There are several reports of  $\alpha$ SYN presence in the mitochondrial outer membrane in both PD mouse models as well as human *post mortem* PD brain tissue [35–37]. Nonetheless, the precise functions of  $\alpha$ SYN in mitochondria remain disputed.

## Alzheimer's disease

Neuropathological hallmarks of AD are the presence of extracellular plaques consisting of A $\beta$  and intraneuronal neurofibrillary tangles composing of aggregated tau protein [38,39] (Figure 2). Other characteristics of the disease involve vascular destruction, chronic neuroinflammation and extensive loss of dendrites, axons and neurons leading to brain atrophy [40,41]. Early signs of AD are short term memory loss which progresses to severe dementia as the disease advances [42]. The A $\beta$  peptide is a product of proteolytic cleavage of the transmembrane amyloid precursor protein (APP), where APP is first cleaved by  $\beta$ -secretase, followed by  $\gamma$ -secretase resulting in the release of A $\beta$  [43,44]. The physiological functions of APP are not well understood, although it has been proposed to be involved in synapse formation, plasticity and migration as well as learning and memory [45–49]. Tau is a neuronal protein that is responsible for microtubule stability and assembly. Tau is dephosphorylated

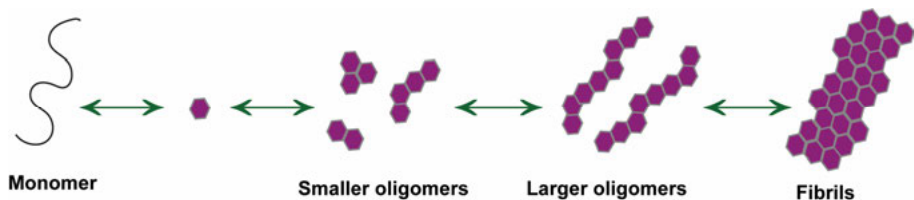
in order to interact with a microtubule and phosphorylated to dissociate from the microtubule. In addition to aggregation, hyper-phosphorylation of tau is also involved in AD pathology [50]. Familial AD account for less than 5% of all cases and are caused by mutations in the genes for APP (*APP*), presenilin 1 (*PSEN1*) and presenilin 2 (*PSEN2*) [51].



*Figure 2.* Presence of phosphorylated tau (unfilled arrow) and A $\beta$  plaques (filled arrow) in a *post mortem* AD brain stained with anti-ptau antibody (green) and anti-A $\beta$  antibody (orange). Scale bars = 20  $\mu$ m. These images were kindly provided by Tobias Mothes.

## A $\beta$ and $\alpha$ SYN aggregation

Depending on the  $\gamma$ -secretase cleavage site, various lengths of A $\beta$  peptides are formed. Monomeric A $\beta_{40}$  is mainly produced, followed by A $\beta_{42}$ . Increased hydrophobicity of A $\beta_{42}$  due to the additional two hydrophobic aa enhances the peptide's aggregation propensity [52–55]. Upon accumulation of high concentrations of A $\beta_{42}$ , this peptide gives rise to the formation of soluble aggregates, referred to as oligomers. Further aggregation of the oligomers produces A $\beta$  fibrils (A $\beta$ -F), which deposit and form plaques [56–58]. Similar to A $\beta$ ,  $\alpha$ SYN undergoes certain conformational changes that increases its aggregation ability. The disordered  $\alpha$ SYN monomers self-assemble and aggregate to soluble oligomeric species, which eventually aggregate further to fibrils [59–62] (Figure 3). Moreover,  $\alpha$ SYN undergoes various post-translational modifications (PTM), including phosphorylation and nitration, which have been linked to enhanced aggregation of  $\alpha$ SYN [63–67]. Phosphorylation (at position ser129) of  $\alpha$ SYN has been extensively studied and staining of *post mortem* brain tissue indicates that a majority of LBs and LNs stain positively for phosphorylated  $\alpha$ SYN [68].



*Figure 3.* Monomeric  $\alpha$ SYN and A $\beta$  adopt a confirmation that promotes aggregation of the protein to oligomers and fibrils.

Over the years, there has been a debate regarding which form of  $\alpha$ SYN and A $\beta$  aggregates that are the most toxic species. The fibrillary form of both proteins were at first believed to be neurotoxic as neurons in close vicinity to  $\alpha$ SYN-F and A $\beta$ -F showed signs of apoptosis [69–72]. Fibrils of both proteins are capable of activating glial cells in the brain via different receptors and thereby initiating an inflammatory response [73–76]. Moreover, fibrils have been suggested to play an important role for cell-to-cell propagation as well as seeding of  $\alpha$ SYN and A $\beta$  [42,77]. During recent years the focus has mainly shifted towards the soluble oligomeric forms of both proteins. Oligomers have been demonstrated to be neurotoxic [53,78–82] by permeabilizing membranes, which in turn leads to intracellular calcium dysregulation and eventually apoptosis [83,84]. Moreover,  $\alpha$ SYN oligomers have been shown to cause mitochondrial damage and lysosomal leakage [85,86].

## Treatments for Parkinson’s disease

Currently, there is no cure for PD and the available drugs only alleviate the symptoms. Although neuronal cell loss can be observed in several regions in the PD brain, there is a good correlation between motor symptoms and dopamine loss [87]. Therefore, many pharmaceutical agents have focused on the replacement of dopamine (i.e. levodopa), dopamine receptor agonists and catechol-o-methyl-transferase (COMT) inhibitors. Levodopa was discovered in the 1960s and remains the most potent drug for alleviating PD symptoms. Upon oral administration, Levodopa is quickly decarboxylated to dopamine. This quick decarboxylation results in only a small portion of the drug reaching the brain [88]. Several ways have been developed to increase the availability of levodopa to the brain, such as COMT inhibitors, which enhance the transport of levodopa across the blood brain barrier (BBB). However, long term usage of levodopa causes side effects such as increased dyskinesia. Therefore, administration of levodopa is usually delayed until the motor symptoms are severe enough to interfere with the patient’s daily life [88,89].

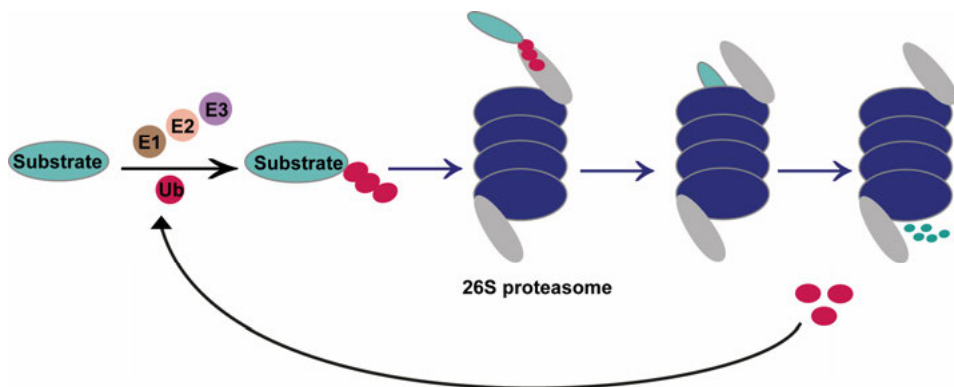
Ongoing research is focusing on finding therapeutic agents that can halt the disease progression. Immunotherapy has proceeded as a promising tool for treatment of neurodegenerative disorders, including AD [90] and possibly also

PD. Passive immunization, using  $\alpha$ SYN-specific antibodies, have shown increased clearance of  $\alpha$ SYN aggregates both in *in vivo* and *in vitro* models [91,92]. Another idea is to prevent  $\alpha$ SYN aggregation or enhance cellular degradation pathways which target  $\alpha$ SYN. Prolyl oligopeptidase (PREP) is a serine protease expressed throughout the body. In the brain, PREP is highly expressed and active in cortical and striatal neurons [93,94]. PREP cleaves peptides smaller than 3 kDa [95]. *In vitro* studies have shown that PREP can interact with  $\alpha$ SYN and increase its aggregation [96]. However, this interaction cannot be mediated by  $\alpha$ SYN cleavage as  $\alpha$ SYN is too large to be a substrate for PREP. Furthermore, PREP has been found to co-localize with  $\alpha$ SYN in *post mortem* PD brain, indicating a possible role of PREP in  $\alpha$ SYN pathology [97]. PREP has been observed to interact with other proteins beyond its enzymatic ability, proposing PREP as a protein interactor [98]. Inhibition of PREP by KYP-2407, a small molecule inhibitor, has been shown to increase  $\alpha$ SYN degradation and prevent  $\alpha$ SYN aggregation both *in vivo* and *in vitro* [99–103]. These findings, suggest KYP-2407 as a potential therapeutic target for PD that needs to be studied further.

## Intracellular degradation pathways

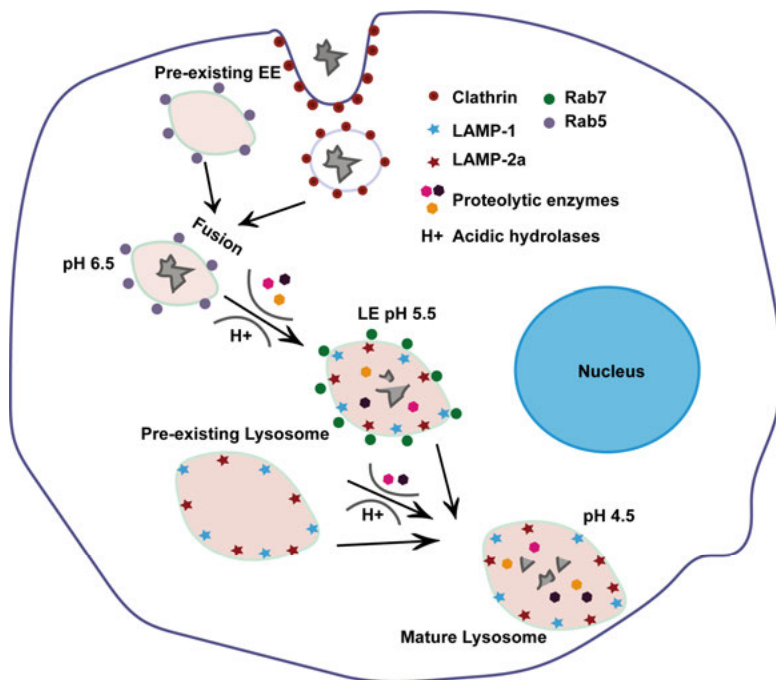
There are two major intracellular degradation pathways: the ubiquitin-proteasome pathway (UPP) and the autophagy-lysosomal pathway (ALP).

The UPP is mainly responsible for degradation of short-lived intracellular proteins. In the UPP system, ubiquitin molecules are covalently bound to a protein in a cascade reaction that requires adenosine triphosphate (ATP). The ubiquitination acts as a recognition signal for the enzyme 26S proteasome, which will degrade the protein [104] (Figure 4).



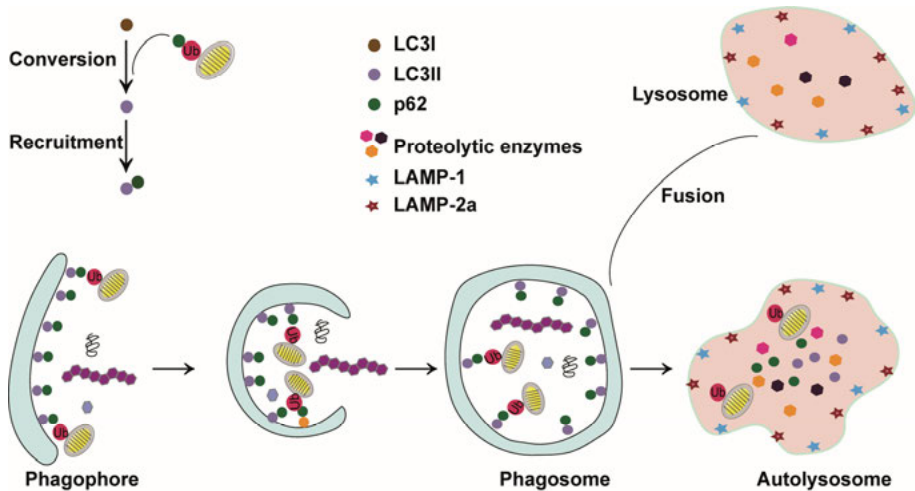
*Figure 4.* The substrate is ubiquitinated in an ATP dependent manner by the enzymes E1, E2 and E3 and recognized by the 26S proteasome, which de-ubiquitinates the substrate and degrades it.

The lysosomal pathway is responsible for degradation of ingested extracellular substrates that enter the endosomal pathway, as well as substrates of the autophagy pathway [105]. The endosomal pathway involves four maturation steps in order to achieve successful degradation of the phagocytosed material. The initial step involves internalization of the material in clathrin-coated pits that bud into vesicles derived from the plasma membrane. These vesicles can either fuse together and form early endosomes (EEs) or fuse with pre-existing EEs facilitated by the GTPase Rab5 [106]. The EEs are mainly located in the periphery of the cell and have a slightly acidic pH (6.5). In the second and third step, EEs mature to late endosomes (LEs) through conversion of Rab5 to Rab7 and the LEs are transported towards the nucleus resulting in a perinuclear localization [107]. Recruitment of acid hydrolases to the interluminal space results in a lower pH (5.5) in LEs [108]. Lysosomal-associated membrane protein 1 (LAMP-1) and 2a (LAMP2a) as well as proteolytic enzymes are also recruited to the LEs. Digestion of the ingested material starts to occur already at this step, due to the presence of proteolytic enzymes and the acidic environment. At the last step, LEs fuse with already existing lysosomes with a lower pH of 4.5, resulting in complete degradation of the material [109,110] (Figure 5).



*Figure 5.* Phagocytosed material is situated in a clathrin-coated vesicle, which fuses with a pre-existing EE and matures further into an LE, via recruitment of acidic hydrolases as well as proteolytic enzymes. The LE fuses with a pre-existing lysosome, leading to degradation of the material.

The autophagy system is divided into the following subgroups: macro-autophagy, chaperone-mediated autophagy (CMA) and micro-autophagy. Macro-autophagy targets larger molecules, such as aggregated proteins and organelles, including mitochondria, which in turn is referred to as mitophagy. Formation of autophagosomes is tightly regulated by autophagy-related proteins, including microtubule associated protein 1 light-chain 3 (LC3) [111]. The phagophore surrounds the material to be degraded, and then elongates and encapsulates the material. Lipidation and conversion of LC3I to LC3II and subsequent recruitment of it to the autophagosome play important roles in the elongation and enclosure of the phagophore to an autophagosome [112]. The UPP is linked to autophagy through p62 in several ways. p62 has a LC3 binding region and transports LC3II to the autophagosome. Furthermore, p62 ubiquitinates and recruits organelles, such as mitochondria, to the autophagosome [113]. Once the autophagosome is formed, it fuses with a lysosome and forms the autolysosome where degradation of the cargo as well as of LC3II and p62 occur [114,115] (Figure 6).



*Figure 6.* Autophagy degrades proteins, aggregated proteins and organelles. Conversion of LC3I to LC3II is important for the autophagosome formation. Recruitment of LC3II as well as organelles to the phagophore depend on p62. Both LC3II and p62 are degraded in the autolysosome.

The CMA pathway degrades specific proteins with a KFERQ motif, which is recognized by the heat shock- protein 70 (Hsp70) in the cytosol. The complex of Hsp70 and the protein of interest is then transported to the lysosomes. Internalization of the protein to the lysosomes requires multimerization of LAMP2a as well as unfolding of the protein by Hsp70. Once the substrate has entered the lysosomal lumen it is degraded by the lysosome [116,117].

Micro-autophagy refers to degradation of cargos directly via the lysosomes, where the lysosomal membrane internalizes the material. However, the mechanisms of micro-autophagy remain largely unknown.

## Degradation of $\alpha$ SYN and A $\beta$

Intracellular and extracellular levels of  $\alpha$ SYN and A $\beta$  are crucial for the aggregation process. Hence, the cell needs to tightly regulate synthesis, degradation and secretion. Many lines of evidence indicate that diminished degradation of  $\alpha$ SYN and A $\beta$  results in elevated levels of the proteins [118,119]. Various mechanisms are involved in the clearance of  $\alpha$ SYN and A $\beta$ , including degradation by proteases as well as intracellular degradation pathways. Proteases that clear both  $\alpha$ SYN and A $\beta$  are for example the matrix metalloproteases [120,121]. In addition,  $\alpha$ SYN is degraded by calpains and neurosin [122,123], whereas A $\beta$  is cleared by insulin-degrading protein and neprilysin [124–126]. The endo-lysosomal pathway as well as ALP have been reported to be involved in degradation of internalized extracellular  $\alpha$ SYN and A $\beta$  aggregates [127–132]. Intracellular  $\alpha$ SYN in neurons has been linked to both UPP and ALP [133,134]. In addition, presence of the KFERQ motif in the  $\alpha$ SYN sequence makes it a target for CMA degradation. However, since the folding stage of the protein is important in CMA degradation, large aggregates are difficult to degrade via this pathway [135]. Aggregated  $\alpha$ SYN impairs cellular degradation pathways by either directly interacting with key enzymes or by affecting other molecules required for the functionality of UPP and ALP [136–139]. For example, *in vitro* studies indicate that  $\alpha$ SYN inhibits the UPP by directly binding to the 26S proteasome or by decreasing levels of ATP by inducing mitochondrial impairments [82,140]. Furthermore, chronic inhibition of proteasomes has been found to negatively affect the autophagy pathway, as these mechanisms are connected [113,141].

## Cellular effects of pathological $\alpha$ SYN

Data collected from many studies demonstrate that aggregation and accumulation of  $\alpha$ SYN has a negative effect on cellular organelles such as the endoplasmic reticulum (ER), Golgi apparatus and mitochondria [85,142–146]. Cultured cells overexpressing  $\alpha$ SYN show an upregulation of ER related stress markers. Furthermore, overexpression of  $\alpha$ SYN in yeast models causes blockage of ER-Golgi trafficking [147]. Golgi-fragmentation is another phenomenon observed in neuronal cell cultures overexpressing  $\alpha$ SYN [143]. However, the effects of Golgi fragmentation are not fully understood.

Mitochondrial dysfunction has been strongly linked to PD progression, especially as three of the genes causing familial PD are involved in mitochondrial functions [148]. Impairment of mitochondrial fusion-fission balance, with a shift towards fission, has been associated with overexpression of  $\alpha$ SYN in neurons, both dependently and independently of the fission protein, DRP-1 [149,150]. Moreover, several studies have reported that disruption of mitochondrial membranes by  $\alpha$ SYN results in the release of reactive-oxygen species (ROS) and neuronal cell death [145,146]. Due to the large distance between the neuronal processes and the cell soma, neurons need mitochondrial presence in their axons. Impaired mitochondrial trafficking from the cell soma to the axons is observed in neurons overexpressing  $\alpha$ SYN. This has been linked to an interaction of  $\alpha$ SYN with cytoskeleton proteins, such as actin and microtubule, which may alter mitochondrial trafficking [78,151–154].

## Alpha-synuclein propagation

Increased intracellular protein levels of  $\alpha$ SYN can cause increased secretion and spreading of  $\alpha$ SYN pathology [155]. Spreading of  $\alpha$ SYN pathology in interconnected anatomical regions of the brain was first suggested by Braak and colleagues in 2003 [156]. The Braak staging hypothesis includes six stages [157]. Alpha-synuclein pathology was suggested to originate from the gastrointestinal tract and then spread via the olfactory tract and vagal nerve towards the olfactory bulb and the brainstem respectively [158]. This hypothesis has gained further support from several studies that confirmed early symptoms, including gastrointestinal problems, loss of smell and sleeping disturbances, in PD patients [159–161]. The presence of  $\alpha$ SYN aggregates in grafted neurons in the brain of PD patients gave rise to the idea that  $\alpha$ SYN might behave as a prion-like protein [162,163]. Once internalized, the  $\alpha$ SYN aggregates act as a template to misfold the endogenous  $\alpha$ SYN of the recipient cell. Besides a passive transfer of  $\alpha$ SYN from dead neurons to the surrounding cells, the question was asked whether cells in the brain actively spread  $\alpha$ SYN aggregates to each other. Two different cellular spreading mechanisms have been proposed for  $\alpha$ SYN transfer between cells: direct cell-to-cell contact and secretion of vesicles containing  $\alpha$ SYN or release of free  $\alpha$ SYN. Tunneling nanotubes (TNTs) were first discovered in 2004, as extensions of the cell cytoskeleton, enabling distal and close contact with neighboring cells [164] (Figure 7). Cellular stress such as exposure to UV light or  $H_2O_2$  has been shown to induce formation of TNTs between cells [165,166]. Transfer of various proteins, ions, organelles as well as microbes by TNTs have been suggested [164,167]. The TNTs are formed through actin polymerization, and involvement of microtubule occurs upon transfer of organelles [168]. However, the intracellular and extracellular signaling pathways that regulate TNT formation remains to be elucidated. In the presence of  $\alpha$ SYN aggregates, neurons and

glial cells have been demonstrated to form more TNTs than untreated cells, confirming that  $\alpha$ SYN induces cellular stress [169–172]. Furthermore, studies have reported transfer of  $\alpha$ SYN via TNTs between neurons and neuron-glia co-cultures [169,170]. These studies suggest TNTs as a propagation mechanism for  $\alpha$ SYN.



*Figure 7.* Actin polymerization enables the formation of the TNT, which is an extension of the cellular cytoskeleton. Cells transfer proteins and ions, as well as organelles via TNTs.

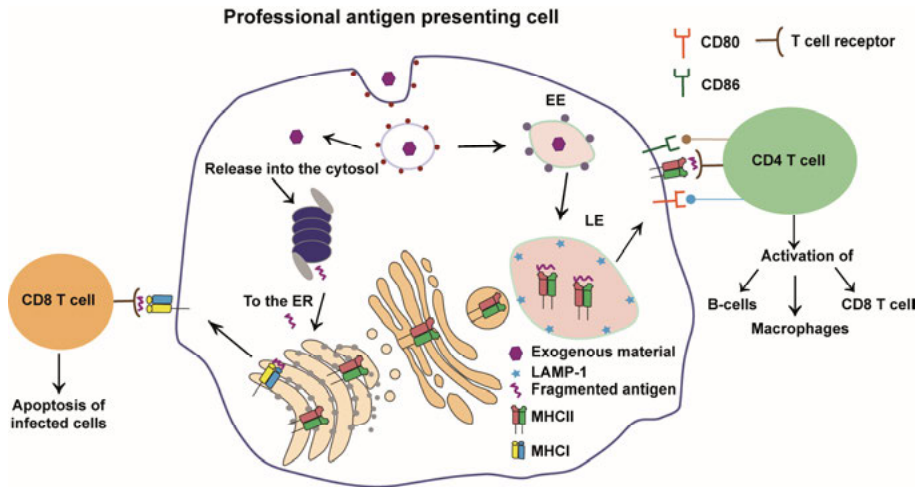
Currently, TNTs are detected using staining for plasma membrane and actin proteins. Lack of specific markers for TNTs has made it difficult to detect them in human or mouse brain sections, which has led to the debate whether TNTs are only formed *in vitro*. Recently, few studies have detected the existence of TNT-like structures *ex vivo* and *in vivo* [173–175]. However, more *in vivo* studies are required to confirm the presence of TNTs in the pathological brain.

## Peripheral immune system

Inflammation is a key biological process in response to pathogens and injury. Cells in the innate immune system, such as neutrophils and macrophages, recognize pathogens or danger signals released from necrotic cells through the pattern-recognition receptors (PRR). There are different types of PRRs but the toll-like receptor family (TLRs) is the most well-known. Binding to the PRRs initiates a cascade of signals inside the cell, leading to secretion of inflammatory mediators such as cytokines and chemokines. These mediators are in turn important for the recruitment of more immune cells to the site of infection/injury as well as for increased phagocytosis. The innate immunity acts within minutes and has a broad recognition of pathogens and danger signals [176].

The adaptive immunity consists of T cells and B cells. These cells only recognize specific antigens and react slower in response to the pathogen than the innate immune system. There are several types of T cells that differ regarding how they are activated and by which mechanism they act. Cytotoxic T cells ( $CD8^+$ ) are activated by the major histocompatibility class I (MHC I) pathway and cause apoptosis in infected cells.  $CD4^+$  T helper-cells are stimulated via the MHC II pathway and are capable of activating  $CD8^+$  T cells and macrophages, as well as B cells. In order to be activated, T cells need to be presented to the antigen. Professional antigen-presenting cells (APCs) are the bridge

between the innate and the adaptive immunity. They engulf the pathogen, which they, in contrast to professional phagocytes, do not completely degrade, but digest into smaller fragments. Professional APCs are capable of expressing both MHC I, which is expressed by almost all cell types, and MHC II that is only expressed by APCs. Hence, they are capable of cross-presentation. After internalization, the material can be sent to the endo-lysosomes or be released into the cytosol. Once released, the UPP digests the cargo to 8-10 aa long fragments that are transported to the ER where the fragments bind to MHC I. The antigen/MHC I complex is then transported to the cell surface where it activates CD8<sup>+</sup> T cells. In endo-lysosomes, the material is degraded into 12-24 aa long pieces. MHC II molecules are transported from the ER via the Golgi apparatus to the LEs where the MHC II binds to the antigen and is transported to the surface. At the surface, CD4<sup>+</sup> T cells bind to MHC II and the antigen. For stimulation of CD4<sup>+</sup> T cells, co-stimulatory molecules including CD80 and CD86 are also required. Once activated, CD4<sup>+</sup> T cells can in turn activate CD8<sup>+</sup> T cells, macrophages and B-cells [176] (Figure 8).



*Figure 8.* Professional APCs are capable of cross-presenting exogenous antigens via the MHC I and MHC II pathway, leading to activation of both CD4<sup>+</sup> and CD8<sup>+</sup> T cells. CD4<sup>+</sup> T cells can, in turn, activate B cells, macrophages and CD8<sup>+</sup> T cells.

## Neuroinflammation

Neuroinflammation refers to inflammation in the nervous system. Due to the BBB, a lack of lymphatic drainage and an alleged lack of professional APCs in the brain, it has been debated whether and how the peripheral immune system and the brain communicate. However, over the past decade studies have suggested that CSF can be drained to the cervical lymphatics, raising the possibility of proteins and other molecules from the brain to come in contact with

the peripheral immune system [177]. CD4<sup>+</sup> T cells have been detected in the CSF, suggesting that these cells “patrol” the brain [178]. In addition, resting macrophages have been shown to be present in choroid plexus as well as in the perivascular space and meninges of the brain [179,180]. The brain itself contains glial cells, including astrocytes and microglia that have immune functions [181,182]. Although inflammation is crucial for the removal of dead cells as well as antigens, a state of chronic inflammation can cause more cell death and thereby become a driving force for the pathology.

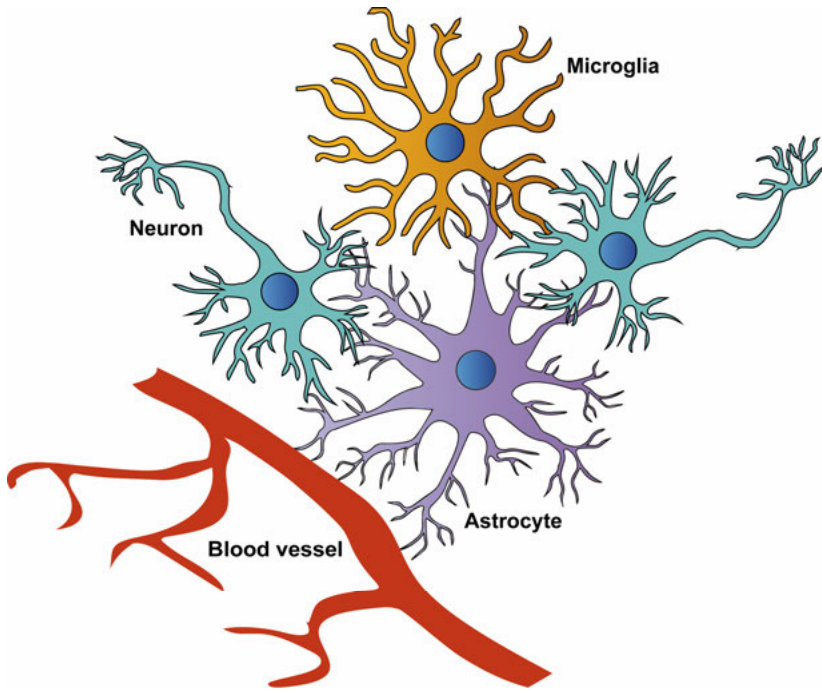
## Glial cells

Glial cells account for almost 50% of the cells in the brain and are divided into different subtypes, including astrocytes and microglia [183]. Astrocytes are the most numerous glia cell type in the brain and were long described as the star-shaped cells that support neurons. Today we have extensive, although not complete, knowledge about their many important functions in both the healthy and diseased brain. In the physiological situation, astrocytes have a remarkable organization, enabling them to have contact with neurons, microglia, other astrocytes and endothelial cells in blood vessels (Figure 9). These contacts allow astrocytes to be involved in synaptic plasticity, neurotransmitter recycling, neuronal maturation, removal of synapses, ion homeostasis and BBB maintenance [184–189]. There are various subtypes of astrocytes in the brain, with distinct morphological differences as well as localization. In addition, the various astrocyte subtypes have different expression profiles of the major astrocytic marker used today: glial fibrillary acidic protein (GFAP) [190].

Unlike astrocytes, which have a neuronal origin, microglia have a myeloid origin and are therefore referred to as brain macrophages. Although researchers agree on the myeloid origin of microglia, there has been an extensive debate regarding which macrophages they derive from. One of the hypotheses is that microglia derive from yolk sac macrophages that migrate to the brain during embryonic development [191]. In the brain, microglia are crucial for neuronal development and survival, synapse pruning and neuronal plasticity. Additionally, microglia are motile cells and are constantly sensing the environment with their processes [192,193].

Upon injury or in disease, astrocytes and microglia respond through a process called reactive gliosis, a defense mechanism against pathology. During this process, both cell types become inflammatory and secrete inflammatory mediators, such as cytokines and chemokines. Furthermore, both cell types are capable of phagocytosis and clearance of pathogens. Activated astrocytes increase in size and upregulate the expression of various cytoskeleton proteins, including GFAP, nestin and vimentin [184]. Activated microglia adopt a more swollen

morphology with shorter processes, often described as amoeboid-like [194]. Gliosis is important for the initiation of an inflammatory response, which can remove pathogens or dead cells [181,182]. However, the consequences of a prolonged gliosis observed in neurodegenerative disorders such as PD and AD, where the pathology progresses for decades, are not yet fully understood.



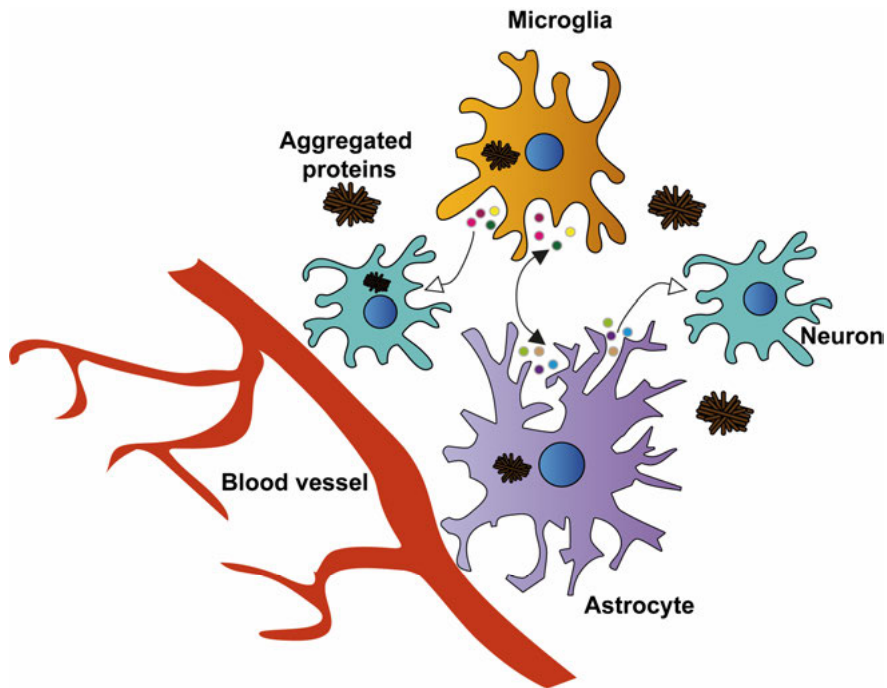
*Figure 9.* Astrocytes have contact with many cells in the brain, including neurons and endothelial cells in blood vessels. These contacts enable them to regulate many processes crucial for the brain homeostasis.

## Chronic neuroinflammation in Parkinson's disease

Although oligomeric forms of  $\alpha$ SYN and A $\beta$  are considered to be the most neurotoxic species of these two proteins [53,78–84], fibrillary forms seem to play an important role in the ongoing neuroinflammation in PD and AD. Data collected from several studies, suggest that the  $\beta$ -sheet structure of fibrils are of great importance for the initiation of an inflammatory response [195,196]. Interestingly, both microglia and astrocytes respond to  $\alpha$ SYN and A $\beta$  fibrils through PRRs, resulting in an inflammatory response [73–76,197–199]. Since  $\alpha$ SYN fibrils are present in the brain for many years, glial activation could be going on for long periods of time. Studies have reported that chronic gliosis prevents astrocytes and microglia from performing their physiological functions, which in turn could be neurotoxic. Furthermore, continuous exposure of

neurons to inflammatory mediators released by glial cells could be neurotoxic [200–206] (Figure 10). Microglia and astrocyte cross-talk is another important aspect of neuroinflammation as these two cell types can stimulate or inhibit each other through secretion of different factors [207–210]. This area has however not been extensively studied and requires further investigation.

An ongoing inflammation, presence of aggregated  $\alpha$ SYN in the CSF and disruption of the BBB can result in recruitment and infiltration of peripheral immune cells to the brain [179,211]. Recently,  $\alpha$ SYN was revealed to have two MHCII binding sites, suggesting that  $\alpha$ SYN might be presented to CD4<sup>+</sup> T cells via the MHCII pathway [212]. Interestingly, the same study showed that CD4<sup>+</sup> T cells derived from the blood of PD patients reacted against fibrillary and phosphorylated  $\alpha$ SYN [212]. These data suggest that although  $\alpha$ SYN is a self-antigen, the body recognizes fibrillary or modified  $\alpha$ SYN as an altered self-antigen or danger signal that the immune system reacts against. In support of this, infiltration of CD4<sup>+</sup> T cells has been observed in *post mortem* brain tissues of PD patients, as well as in PD mouse models [211,213,214]. However, how T cells act in the brain and which cells they interact with is unknown.



*Figure 10.* Presence of aggregated proteins activate the glial cells in the brain, leading to secretion of inflammatory mediators, which is important for the glial cross-talk (filled arrows). Pro-longed activation of the glial cells can lead to dysfunctional glial cells that cannot perform their physiological functions. Also, chronic secretion of inflammatory factors can be neurotoxic (unfilled arrows).

# Aims

The overall purpose of this thesis was to examine how astrocytes and microglia are involved in  $\alpha$ SYN pathology, focusing on degradation, cell-to-cell propagation and inflammation.

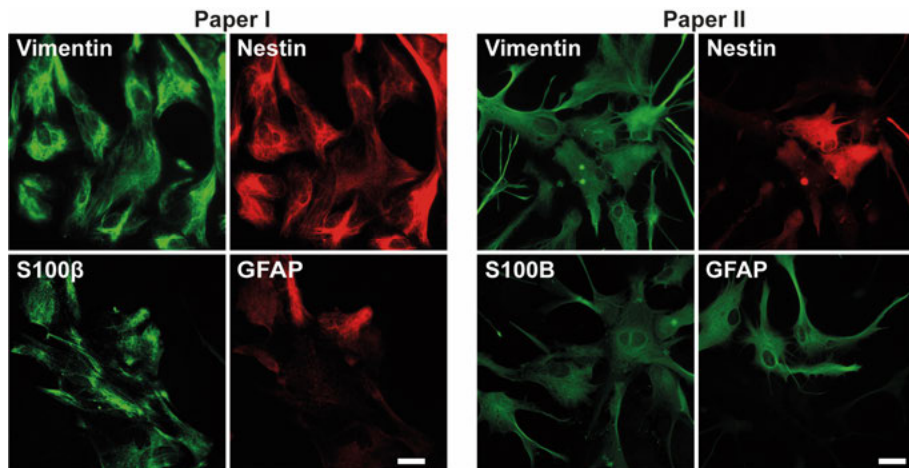
## Specific aims:

- I To investigate uptake, degradation and cell-to-cell transfer of  $\alpha$ SYN by human astrocytes.
- II To examine whether human astrocytes and microglia have the capacity to behave as APCs in the presence of  $\alpha$ SYN aggregates.
- III To analyze how astrocytes and microglia communicate with each other in the presence of aggregated  $\alpha$ SYN and A $\beta$ .
- IV To study if inhibition of PREP increases degradation of  $\alpha$ SYN-F.

# Methods

## Cell culture models

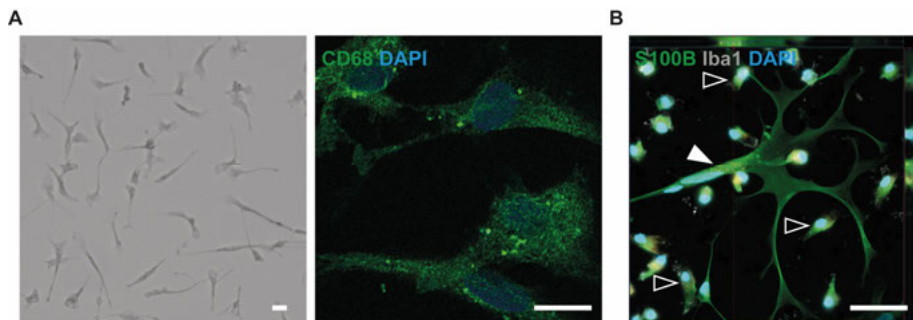
In order to prevent and treat a disease, understanding its molecular mechanisms is of great importance. *In vitro* models are simplified simulations of an organ and allow us to study one mechanism or pathway at a time. Cell culture models have been central to all the studies included in this thesis. In **Paper I and II**, astrocytes were generated from human embryonic stem cells (ESC), which were driven towards a neuronal fate prior to astrocyte differentiation and maturation. In **Paper I**, 1% fetal bovine serum (FBS) was used to promote maturation of the astrocytes [215] (Figure 11). In order to study  $\alpha$ SYN propagation between  $\alpha$ SYN treated astrocytes and untreated astrocytes, a co-culture setup of unlabeled astrocytes and RFP labeled astrocytes was used [215]. In **Paper II**, 20ng/mL of ciliary neurotrophic factor (CNTF) was used instead of FBS (Figure 11). Studies comparing these two factors have shown that FBS gives rise to a higher degree of reactive astrocytes compared to CNTF [216]. Astrocytes from 90-120 days *in vitro* were used in both studies.



*Figure 11.* Characterization of FBS derived astrocytes, used in Paper I and CNTF derived astrocytes, used in Paper II, using different astrocytic markers. Scale bar = 20 $\mu$ m.

Adult human microglia were isolated from the temporal lobe of patients undergoing surgery due to epilepsy in **Paper II** (Figure 12A). The isolation involved several steps: separation of blood vessels, tissue dissociation using enzymes, tissue homogenization and finally Percoll gradient to separate microglia from myelin and other cell types [217]. Adult human microglia were cultured up to three weeks *in vitro*.

In **Paper III**, astrocytes and microglia were derived from the same human induced pluripotent cell (iPSC) line in order to avoid immunological reactions when the two cell types were studied in co-cultures. To enable formation of astrocytes, iPSC cells were differentiated to neuroepithelial-like stem (NES) cells, which are the neural stem cells giving rise to cells of neural origin [218]. The NES cells, were then differentiated to astrocytes and matured, using the following four factors: insulin-like growth factor 1 (IGF-1), activin A, basic fibroblast growth factor (bFGF) and heregulin for 28 days [219] (Figure 12B). Microglia, on the other hand, originate from hematopoietic stem cells (HPCs). HPCs were derived from iPSCs, before differentiation to microglia cells, and were validated using flow cytometry analysis. Microglia were generated using the following factors: IL-34, macrophage colony-stimulating factor (MCSF), IDE1, CX3CL-1 and CD200 [220] (Figure 12B). Both cell types were used for experiments directly after the differentiation protocol was completed. In **Paper III**, we used three cell culture models: monoculture of astrocytes, monoculture of microglia and co-culture of both cell types. For the co-culture system, microglia maturation medium together with all the factors required for both astrocytes and microglia was used.



*Figure 12.* Adult human microglia from epilepsy patients undergoing surgery shown in bright-field and stained for CD68 (A). Co-culture system of iPSC derived astrocytes (filled arrows) and microglia (unfilled arrows) (B). Scale bar in A = 20 $\mu$ m and in B = 10 $\mu$ m.

In paper **IV**, the human neuroblastoma cell line SH-SY5Y, and the human astrocytes described in **Paper III** were used.

## Immunocytochemistry

Immunocytochemistry (ICC) was performed to assess the presence of certain antigens in cells. This technique is based on the specific binding of a primary antibody to the antigen of interest. In direct immunostaining, the primary antibody is labeled with a fluorophore that enables detection. In indirect immunostaining, a fluorophore-labelled secondary antibody is used, which binds specifically to the primary antibody. Indirect immunostaining has a higher specificity and enables amplification of the signal, as each primary antibody can be detected by several secondary antibodies. In this thesis, we have used indirect immunostaining with fluorescence microscopy as a detection method in all four papers. Alexa flour 488/555 Phalloidin and Alexa flour 350/488 WGA were used to stain the cell cytoskeleton and membranes, respectively. Nuclei were stained with mounting medium containing 4', 6-diamidino-2-phenylindol (DAPI) which binds to DNA.

## Immunohistochemistry

In **Paper II**, paraffin embedded human brain sections from mesencephalon and striatum were analyzed with immunohistochemistry (IHC). Similar to ICC, IHC is based on primary antibodies that bind to an antigen of interest, but within tissue. In order to enable the binding, sections needs to be processed by removing the paraffin and “opening up the tissue” through antigen retrieval methods. In **Paper II**, the tissue was boiled in citric buffer (pH=6) and permeabilized before the primary antibody solutions were added. Unlike the traditional immunofluorescence, where the secondary antibody is conjugated with a fluorophore, we used secondary antibodies conjugated with horseradish peroxidase (HRP). The signal was developed using the Tyramide signal amplification (TSA) system. Once fluorophore-labeled tyramide is added to the section, the HRP from the secondary antibodies activates the tyramide, giving rise to a signal. TSA is a sensitive method that allows detection of low expressing antigens.

## Microscopy techniques

Microscopy techniques are central to all papers included in this thesis. Fluorescence microscopy was used to investigate uptake, degradation, and accumulation of toxic protein aggregates in all four studies. In addition to fluorescence microscopy, confocal microscopy was used to verify TNTs and co-localization of  $\alpha$ SYN with various intracellular markers. In a fluorescence microscope, the specimen is illuminated with light resulting in excitation of the entire sample giving rise to an unfocused background. A confocal microscope

excites one focal point at the time and possess a pinhole which eliminates background signal, resulting in a better resolution. In **Paper I**, transmission electron microscopy (TEM) was used to study the effects of  $\alpha$ SYN aggregates on cellular organelles such as mitochondria, ER and Golgi and to visualize TNTs. TEM was also used in **Paper II-IV** for the purpose of characterizing the  $\alpha$ SYN and A $\beta$  fibrils with regards to structure and whether the sonication was successful. The resolution of an image is dependent on the wavelength. The use of electrons, which have a shorter wavelength than light, provides the possibility to study small structures inside the cells, i.e. organelles, in TEM. Time lapse microscopy, which allows live imaging of the cells, was performed to analyze cell-to-cell transfer of  $\alpha$ SYN between astrocytes in **Paper I** and to study how microglia and astrocytes communicated with each other in the presence of  $\alpha$ SYN and A $\beta$  aggregates in **Paper III**.

## Synthetic $\alpha$ SYN oligomers and fibrils

Alpha-synuclein and A $\beta$  have the capacity of self-aggregating in certain circumstances. Two different species of aggregated  $\alpha$ SYN were used in this thesis. In **Paper I**, oligomeric  $\alpha$ SYN was generated by incubating  $\alpha$ SYN monomers with the reactive aldehyde 4-hydroxy-nonenal (HNE) for three days at 37°C. The oligomers were characterized using size exclusion chromatography (SEC-HPLC). In **Paper II-IV**,  $\alpha$ SYN-F was generated by shaking the monomers for seven days at 37°C. The fibrils were evaluated using TEM and fluorescent thioflavin T, which binds to  $\beta$ -sheet structures. Before addition to the cells, the fibrils were sonicated to smaller pieces to enable uptake (Figure 13). Monomeric  $\alpha$ SYN was used as a control to the oligomers and the fibrils in **Paper I and II**, respectively. All the different species of  $\alpha$ SYN were Cy3-labeled using Cy3<sup>AM</sup> antibody labeling kit, which labels the lysine amino acid throughout the protein. In **Paper III**, A $\beta$ -F were generated by shaking the A $\beta$  monomers for four days at 37°C. The fibrils were characterized using TEM and sonicated before being added to the cells (Figure 13).

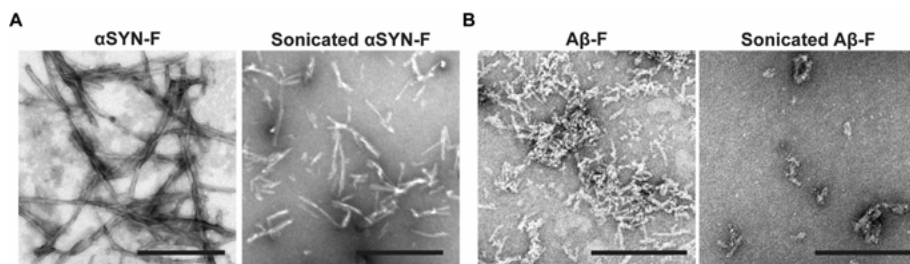


Figure 13. TEM images of  $\alpha$ SYN-F and A $\beta$ F. Scale bar in A = 2 $\mu$ M and in B = 1  $\mu$ M.

## Flow cytometry

Flow cytometry is a broadly used method capable of measuring different properties of a cell population such as their size, granularity, and expression of various markers. This is very helpful in different research areas such as immunology where different immune cell populations can be categorized based on their granularity and size. Cells or particles travel in a fluid stream through a beam of light and their properties are recorded based on how they scatter the light beam and whether they fluoresce or not. Here, the levels of cell surface markers as well as intracellular markers can be quantified using direct immunostaining where the primary antibody, specific to the antigen of interest, is labeled with a fluorophore. In **Paper II**, astrocytes and microglia were analyzed to determine the expression of cell surface molecules required for antigen presentation. Additionally, flow cytometry can be used to assess the purity of a certain cell population. In order to achieve a pure microglia population, we optimized the number of days that were required to differentiate iP-SCs to HPCs by analyzing the purity of the population using HPC specific markers such as CD43 and CD41. In some cases where the population of interest needs to be isolated and concentrated for analysis or *in vitro* cultures, flow cytometry can also be used to isolate that specific population through sorting.

## Western blot analysis

Western blot (WB) analysis allows detection of a protein of interest in samples consisting of many different proteins, such as cell homogenates. In order to separate proteins based on size, the samples are given a negative net charge which makes all the proteins to migrate towards a positive pole during gel electrophoresis. Once the migration is completed, the proteins in the gel are transferred to a polyvinylidene fluoride (PVDF) membrane using an electrical field. A primary antibody specific to the protein of interest is added to the membrane. Using a secondary antibody that is coupled with an HRP molecule, the protein of interest can be detected with chemiluminescence. In **Paper I and IV**, WB analysis was used to confirm the accumulation of  $\alpha$ SYN aggregates in cells by analyzing the cell homogenates for high molecular species of the protein. Furthermore, WB analysis was used to evaluate if  $\alpha$ SYN aggregates affected the cellular degradation pathways.

## Cytokine assay

In **Paper III**, media from  $\alpha$ SYN-F or A $\beta$ -F treated cells were analyzed using cytokine assay, to determine which inflammatory mediators that had been released from the cells. The assay is based on a nitrocellulose membrane that is coated with 36 capture antibodies with specific target proteins. The medium samples were incubated with a mixture of biotinylated detection antibodies before addition to the membrane and the signal was detected with chemiluminescence.

## Enzyme linked immunosorbent assay

Enzyme linked immunosorbent assay (ELISA) is another antibody based technique. Among the different types of ELISA, we have used sandwich ELISA in **Paper III and IV** to measure extracellular  $\alpha$ SYN and A $\beta$  in the cell culture medium. In sandwich ELISAs, plates are coated with an antibody specific to the target protein. After application of the sample to the plate, a second antibody is added, which will also bind to the protein of interest, making a sandwich with the antigen in the middle. The second antibody is often coupled with HRP or biotin enabling detection of the signal.

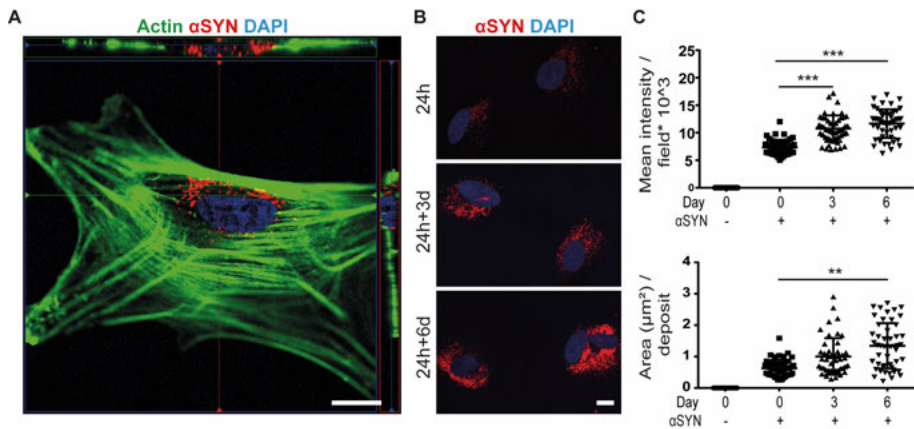
## Image analysis

Analyzing and quantifying microscopy images has been essential to the papers included in this thesis. Using the ImageJ software, macros were developed that enabled the quantification of different parameters including: measurements of intracellular  $\alpha$ SYN in **Paper I, III and IV** and A $\beta$  inclusions in **Paper III**, morphology analysis of mitochondria and ER in **Paper I**, quantification of TNTs in **Paper I**,  $\alpha$ SYN and MHCII measurements in human brain sections in **Paper II**, quantifications of WBs in **Paper I and IV** as well as quantification of cytokine assays in **Paper III**.

# Results and discussion

## Cellular effects of $\alpha$ SYN oligomers on astrocytes

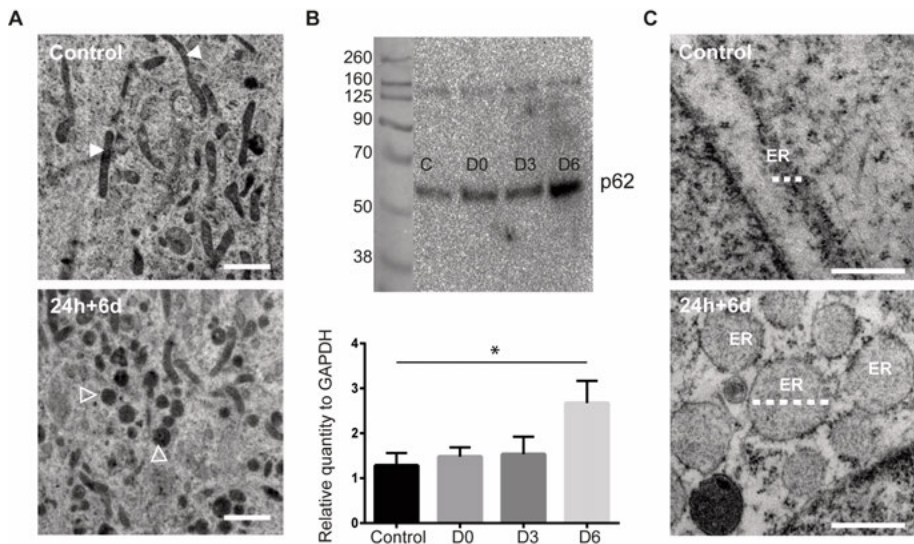
In **Paper I**, human ESC-derived astrocytes were exposed to Cy3-labeled oligomeric  $\alpha$ SYN, in order to examine their role in  $\alpha$ SYN pathology. The reason for this experimental setup is that astrocytes express very low levels of  $\alpha$ SYN. Hence, intracellular deposits of  $\alpha$ SYN in astrocytes in the PD brain are believed to occur by ingestion of extracellular  $\alpha$ SYN released from dying neurons. Already after 24h of exposure we could observe intracellular Cy3-labeled  $\alpha$ SYN oligomers, indicating that the astrocytes had successfully engulfed  $\alpha$ SYN (Figure 14A). To study how the intracellular  $\alpha$ SYN oligomers affect astrocytic health, we removed the  $\alpha$ SYN oligomers from the medium after 24h by washing the cells extensively. Subsequently, the cells were cultured for additional three or six days prior to analysis, i.e. 24h+3d and 24h+6d, respectively. Quantification of the intracellular  $\alpha$ SYN at the different time-points showed that astrocytes were not capable of degrading the aggregates and instead accumulated  $\alpha$ SYN inside the cell (Figure 14B-C).



*Figure 14.* Confocal microscopy image showing that astrocytes engulf  $\alpha$ SYN oligomers during the 24h exposure (A). Quantification of images from all three time points shows that astrocytes store aggregated  $\alpha$ SYN over time (B-C). Scale bars = 20 $\mu\text{m}$ .

Since ingested material usually follows the endo-lysosomal pathway, we stained astrocytes for the endo-lysosomal marker, LAMP-1. Immunostaining revealed a partial co-localization of  $\alpha$ SYN with LAMP-1 at 24h and an increased co-localization at 24h+3d, which was lost by 24h+6d. Thus, the  $\alpha$ SYN oligomers entered the endo-lysosomal pathway, but later escaped, instead of being degraded. To find out in which subcellular localization the aggregates were situated, we performed immunostainings for various organelles, such as mitochondria, ER and Golgi apparatus. Interestingly, the  $\alpha$ SYN aggregates co-localized with trans-Golgi markers at 24h+6d.

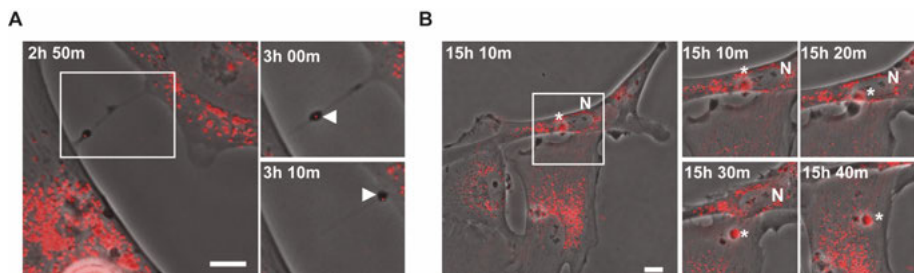
We also observed that the mitochondrial morphology was different in  $\alpha$ SYN treated astrocytes, compared to controls. Using TEM as well as fluorescence microscopy we could demonstrate that there was an increase in fragmented mitochondria in  $\alpha$ SYN treated cells, indicating enhanced mitochondrial fission. Additionally, the  $\alpha$ SYN treated cells exhibited darker mitochondria with diminished cristae (Figure 15A). The mitochondrial fission-fusion dynamic is tightly regulated and essential for the synthesis of new mitochondria through fission and rescue of damaged mitochondria through fusion. Severely damaged mitochondria are supposed to be removed and degraded through a process called mitophagy. Therefore, we investigated whether the accumulation of the fragmented mitochondria could be explained by impaired autophago-lysosomal degradation. Indeed, astrocytes that had been treated with  $\alpha$ SYN, accumulated the autophagosomal protein p62, which is normally degraded when the autophagosome fuses with the lysosome (Figure 15B). Another deviation, noticed with TEM imaging, was swelling of the ER in astrocytes that had accumulated  $\alpha$ SYN (Figure 15C). All in all, these data indicate that the astrocytes were heavily affected by  $\alpha$ SYN accumulation as shown by impaired degradation, imbalanced mitochondrial dynamic and ER stress.



*Figure 15.* Mitochondria morphology are altered in astrocytes that stored  $\alpha$ SYN aggregates: from elongated (filled arrows) to round and small (unfilled arrows) (A). Accumulation of p62 indicates that the autophago-lysosomal pathway is affected (B). Astrocytes treated with  $\alpha$ SYN oligomers display ER swelling (C). Scale bars = 1 $\mu$ m.

## Astrocytes are involved in $\alpha$ SYN propagation

In **Paper I**, it became evident that the astrocytes were severely stressed by the accumulation of  $\alpha$ SYN. As a consequence, the astrocytes formed an increased number of TNTs between each other. Notably, the astrocytes transferred  $\alpha$ SYN to one another through TNTs (Figure 16A) and by direct membrane fusion (Figure 16B). Apparently, smaller inclusions were transferred through TNTs, whereas larger inclusions were transmitted through very close contact and membrane fusion.



*Figure 16.* Astrocytes transfer  $\alpha$ SYN via TNTs (A) as well as direct membrane contact (B), as shown by time-lapse microscopy. Arrows in A indicate the transfer of  $\alpha$ SYN deposit between two astrocytes. Stars in B show  $\alpha$ SYN inclusion being transported from one astrocyte to another via membrane fusion. Scale bars = 10 $\mu$ m.

In order to find out whether affected astrocytes are capable of transferring  $\alpha$ SYN to healthy astrocytes, we co-cultured untreated astrocytes with  $\alpha$ SYN treated astrocytes. Indeed, direct cell-to-cell contact through TNTs was detected between the healthy and diseased astrocytes. Using latrunculin B, which inhibits actin polymerization and TNT formation, we could decrease the  $\alpha$ SYN propagation. Using the co-culture system, we also demonstrated that, in addition to  $\alpha$ SYN, mitochondria were transferred between the astrocytes (Figure 17). Interestingly, healthy astrocytes transferred more mitochondria to diseased astrocytes than vice versa. Taken together, our results indicate a possible role of astrocytes in  $\alpha$ SYN propagation via cell-to-cell transfer and demonstrate that healthy astrocytes are capable of helping other astrocytes in stress by spreading mitochondria.

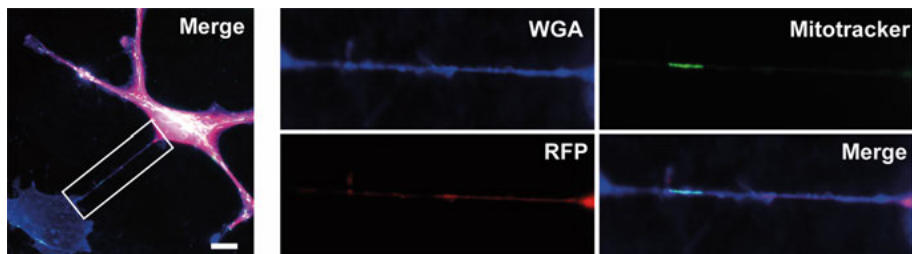
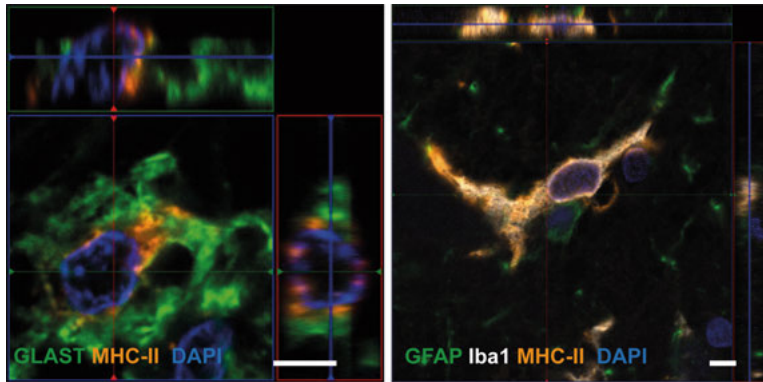


Figure 17. Mitochondria (green) is sent via TNT from healthy astrocytes (red) to  $\alpha$ SYN treated astrocyte. Scale bar = 20  $\mu$ m.

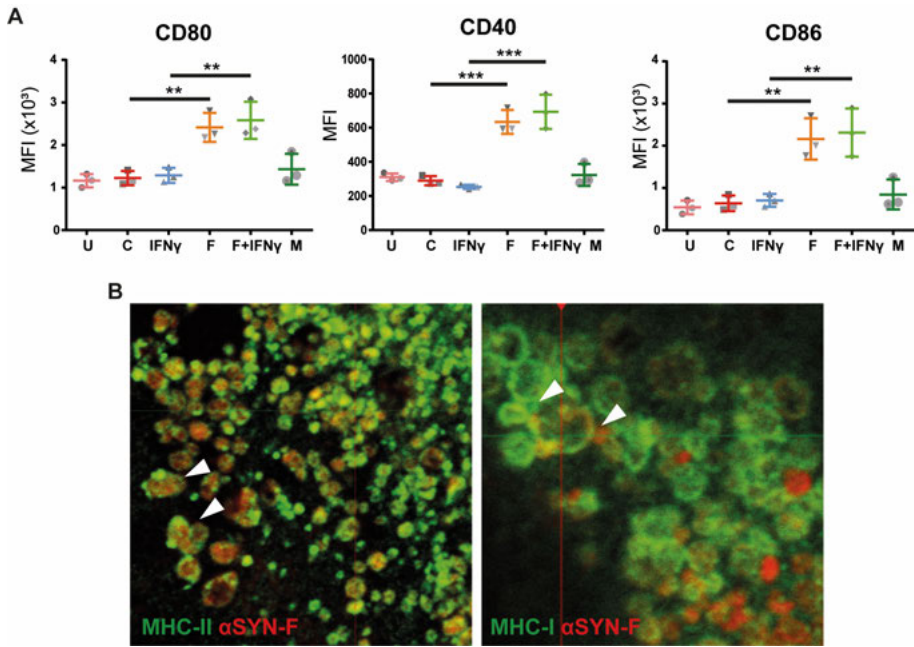
## Are astrocytes a bridge between the brain and peripheral immunity?

Presence of peripheral immune cells in the post mortem PD brain has been shown in several studies [211,213,214], raising the question whether T cells have a role in PD pathology. Moreover, a recent study demonstrated that T cells from PD patients recognize and react to monomeric as well as  $\alpha$ SYN-F [212], proposing that  $\alpha$ SYN has been presented to these T cells. If the T cells enter the brain, which cells do they interact with? Is  $\alpha$ SYN presented to T cells in the brain? If so, which are the antigen presenting cells in the brain? In **Paper II**, in an effort to answer these questions, we stained human PD brain sections and control sections with MHCII, a molecule required for the interaction with CD4<sup>+</sup> T cells. Interestingly, MHCII could be detected in both astrocytes and microglia (Figure 18).



*Figure 18.* Confocal microscopy showing co-localization of MHCII with GLAST (astrocytes) and Iba1 (microglia) in the brain of a PD patient. Scale bars = 5µm.

Professional APCs possess several unique features other than expressing MHCII. First, they are capable of cross-presentation with MHCI and MHCII. Second, they are slow degraders and express molecules essential for T-cell interaction such as CD80, CD86, CD40 and PDL1. To elucidate which of these two cell types might behave as APCs in PD, we treated human astrocytes and microglia with  $\alpha$ SYN-F and analyzed the cells using flow cytometry. Both astrocytes and microglia were capable of expressing MHCII. Notably, only astrocytes expressed CD80, CD86 and CD40 after treatment with  $\alpha$ SYN fibrils, suggesting that astrocytes have the capacity to transform into APCs (Figure 19A). Next, we investigated whether intracellular  $\alpha$ SYN in astrocytes co-localizes with MHCII. Antigen can encounter MHCII in LEs and MHCI in the ER and then travel together as a complex to the cell surface. Immunostaining showed that the majority of  $\alpha$ SYN aggregates were located within the endo-lysosomal pathway and encapsulated by MHCII. However,  $\alpha$ SYN could also be found encircled by MHCI in the periphery of the cell (Figure 19B). These findings suggest that  $\alpha$ SYN could encounter both MHCI and MHCII in astrocytes. We also found MHCII expressing astrocytes surrounding CD4<sup>+</sup> T cells in the brain sections of PD patients. In conclusion, these data suggest that astrocytes have the capacity to interact with T cells and activate them.

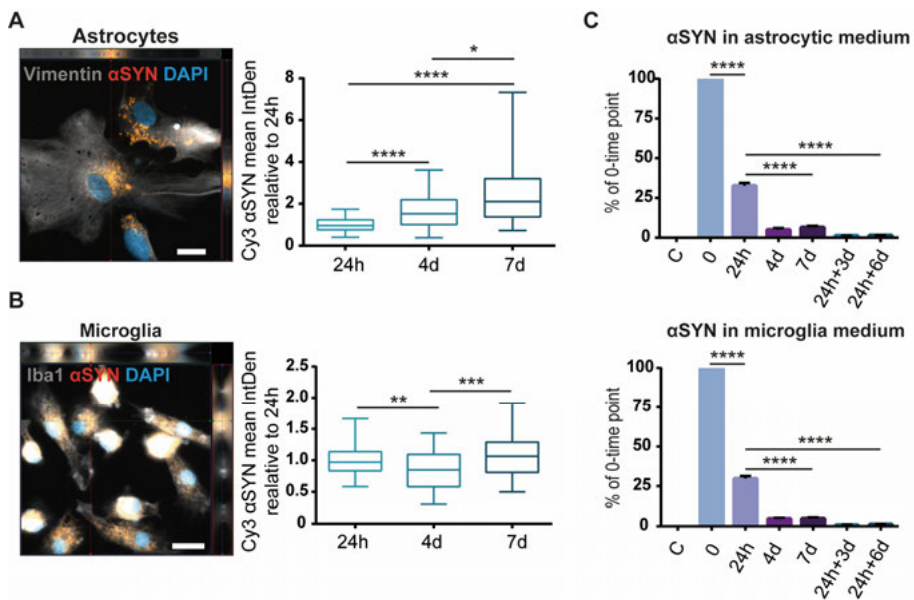


*Figure 19.* Flow cytometry analysis demonstrates that astrocytes express CD80, CD40 and CD86 on their surface following treatment with  $\alpha$ SYN-F (A). MHCII and MHCI surround  $\alpha$ SYN inclusions inside astrocytes (arrows) (B). U = unstained, C = control, F =  $\alpha$ SYN-F and M = monomers. MFI= mean fluorescent intensity.

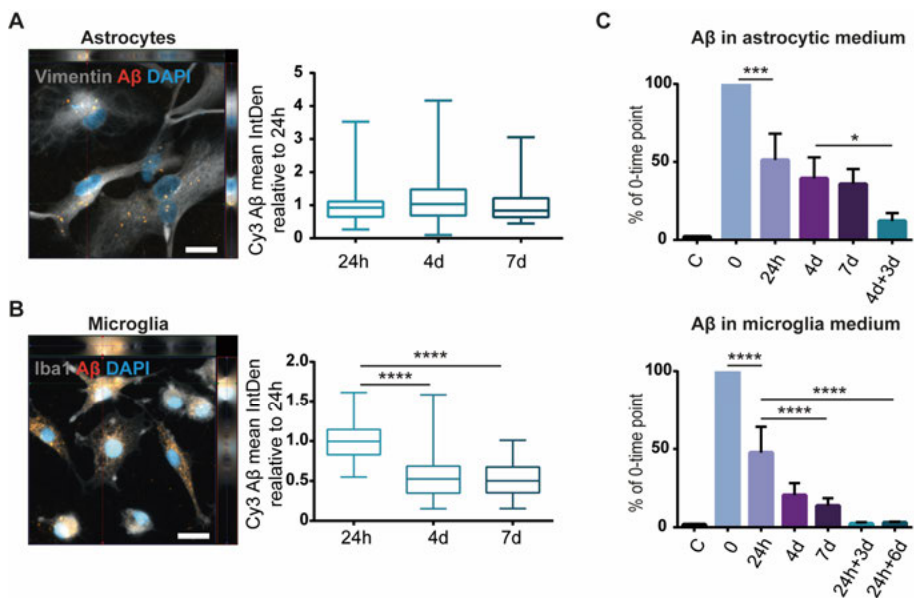
## Microglia are better degraders than astrocytes

In **Paper III**, we aimed to investigate how astrocytes and microglia coordinate their functions to tackle Cy3-labeled  $\alpha$ SYN-F and A $\beta$ -F. In order to answer this question, we also needed to examine how each cell type performed alone. The study design in **Paper III** was composed of two different setups. In the first, the cultures were exposed to a 24 h  $\alpha$ SYN-F or A $\beta$ -F pulse, followed by additional culturing for 3 or 6 days (24h+3d or 24h+6d) (setup 1). In the second setup, the cultures were continuously exposed to  $\alpha$ SYN-F or A $\beta$ -F for 24h, 4 days and 7 days (setup 2). In this and the following section, both  $\alpha$ SYN-F and A $\beta$ -F will be referred to as aggregates/inclusion/deposits, unless the data is only concerning one of the proteins.

At 24h, both cell types appeared to have engulfed similar levels of  $\alpha$ SYN-F indicating that they are equally effective at taking up  $\alpha$ SYN-F. However, microglia seemed to be better at ingesting A $\beta$ -F, compared to astrocytes. In setup 1, the intracellular inclusions were markedly reduced over time in both cell types. In order to examine whether this reduction was due to degradation of the ingested aggregates and not secretion, we performed ELISA on the cell culture media. At 24h+3d and 24h+6d, very low levels of the aggregates could be detected in the medium, indicating that both microglia and astrocytes had successfully degraded a large proportion of the deposits. In setup 2, astrocytes accumulated the  $\alpha$ SYN aggregates, as the Cy3 signal was higher at 7d compared to 24h (Figure 20A). Microglia, on the other hand, had similar levels of intracellular  $\alpha$ SYN inclusions at 7d as 24h (Figure 20 B). Medium analysis revealed that both cell types continuously engulfed  $\alpha$ SYN aggregates during the seven days of treatment as the levels declined from 24h to 7d (Figure 20C). In astrocytes, intracellular A $\beta$  levels were constant over the 7d, while levels in microglia decreased significantly from 24 h to 7d (Figure 21A-B). Medium analysis illustrated that microglia ingested A $\beta$ -F constantly during 7d, while astrocytes did not engulf more A $\beta$ -F after 24h (Figure 21C). Thus, the astrocytes accumulated the engulfed material, while microglia degraded it to some degree. In conclusion, these data suggest that microglia are more effective in degrading  $\alpha$ SYN-F and A $\beta$ -F compared to astrocytes.



*Figure 20.* Astrocytes accumulate  $\alpha$ SYN over time (A), whereas intracellular  $\alpha$ SYN levels in microglia were stable (B). Both cell types clear equal levels of  $\alpha$ SYN-F from the cell culture medium (C). Scale bars = 20 $\mu$ m.



*Figure 21.* Astrocytes store the ingested  $A\beta$ -F (A) whereas microglia degrade the  $A\beta$  aggregates (B). Microglia engulf  $A\beta$ -F continuously over 7d, whereas astrocytes reach a plateau already at 24h (C). Scale bars = 20 $\mu$ m.

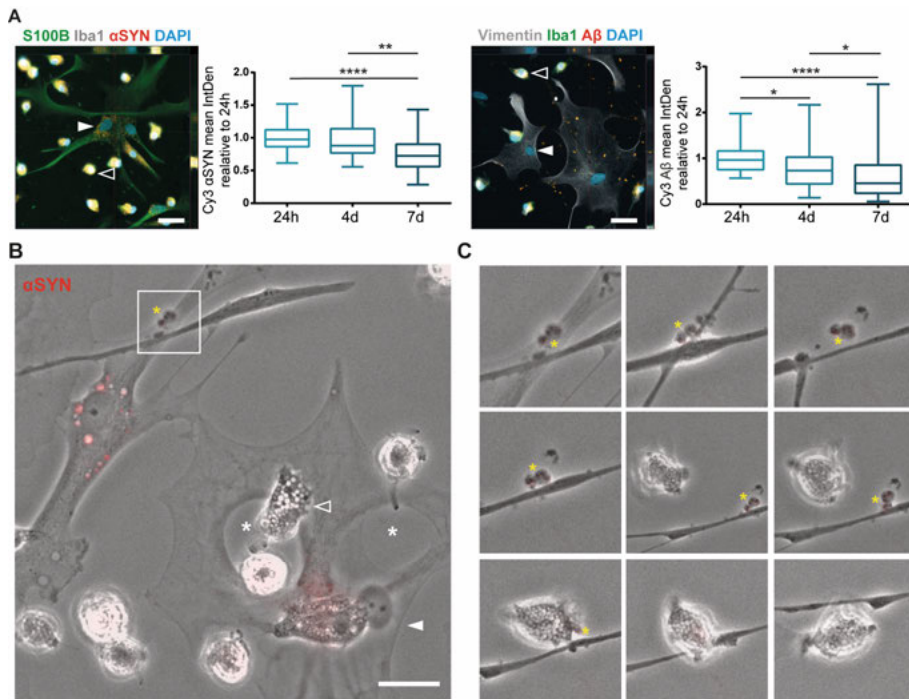
## Astrocytes and microglia store less $\alpha$ SYN and A $\beta$ aggregates when in co-culture

Chronic neuroinflammation is a hallmark in both the PD and AD brain. In the diseased brain, microglia and astrocytes enter an inflammatory state and are believed to be responsible for the inflammatory process. Nevertheless, very little is known about how these two cell types communicate and the impact of their cross-talk in PD and AD. In the first part of **Paper III**, the data indicated that microglia were better degraders than astrocytes. Next, we investigated the interplay between the two cell types when co-cultured, with regards to uptake and degradation of  $\alpha$ SYN-F and A $\beta$ -F. The same two study setups were used as described above for the monocultures.

In setup 1, the co-cultures cleared inclusions as efficiently as the microglial monocultures. Notably, the co-cultures were also capable of degrading the aggregates in setup 2 as the total Cy3 levels of intracellular deposits at 7d were significantly reduced compared to 24h. These were very intriguing results and led to us studying the aggregate content of each cell type in the co-cultures. Image analysis revealed that the intracellular Cy3 signal was reduced in both microglia and astrocytes at 7d. In order to examine whether this was due to elevated degradation or increased secretion, total  $\alpha$ SYN and A $\beta$  levels were examined in the medium. Our analysis showed that the cells indeed degraded the inclusions better in the co-culture system (Figure 22A). The next question to answer was in which way the cellular interplay could result in increased degradation. It is possible that astrocytes and microglia stimulate each other by secretion of various factors and thereby degrade the protein aggregates better. Another possibility is that astrocytes transfer protein aggregates to microglia, as they are better degraders and in some way stimulate microglia to degrade even better.

In order to investigate the second scenario, we analyzed spreading of  $\alpha$ SYN via astrocytic secretion as well as direct cell-to-cell propagation. By adding conditioned media from treated microglia to untreated astrocytes and vice versa, we found that astrocytes secrete more  $\alpha$ SYN compared to microglia. Notably, time-lapse microscopy illustrated that astrocytes release round vesicle-like structures containing  $\alpha$ SYN, which were phagocytosed by microglia (Figure 22B). Fluorescence microscopy revealed various types of cell-to-cell contact between astrocytes and microglia. For example, distal contact via TNTs appeared repeatedly between the two cell types. Direct contact was also observed throughout the culture. Interestingly, microglia were shown to be encircled by astrocytic membrane and contacted the astrocytes with their protrusions (Figure 22B). Altogether, these data indicate that microglia help astrocytes with their  $\alpha$ SYN and A $\beta$  burden. Further studies are required to elu-

cidate the molecular mechanism explaining why microglia degrade the aggregates more efficiently in the presence of astrocytes or if both cell types become better degraders in each other's presence.

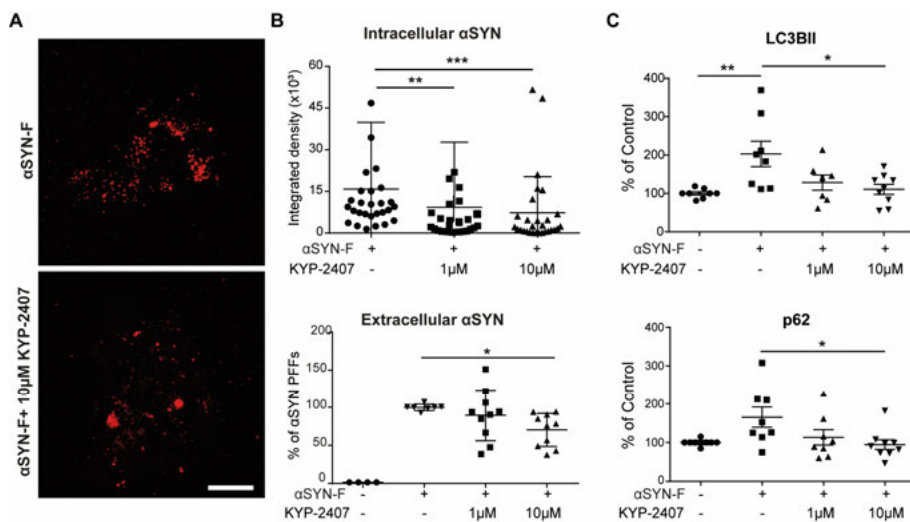


**Figure 22.** Astrocytes (filled arrows) and microglia (unfilled arrows) degrade  $\alpha$ SYN and A $\beta$  aggregates better when in co-culture (A). Microglia and astrocytes have direct contact. The astrocytes surround microglia with their membrane (B white star). Astrocytes release vesicle like structures composed of  $\alpha$ SYN that are engulfed by neighboring microglia (B yellow stars). C is zoomed in images of the white rectangle and yellow star shown in B. Scale bars = 20 $\mu$ m.

## Is KYP-2407 a possible therapeutic drug for PD?

PREP inhibition by KYP-2407 has been shown to increase  $\alpha$ SYN degradation [99,101–103]. However, little is known about the molecular mechanisms by which this inhibitor acts. In **Paper IV**, we studied underlying mechanisms of  $\alpha$ SYN fibril degradation in neuron-like cells. We also aimed to investigate if KYP-2407 has any effects on astrocytic storage of  $\alpha$ SYN aggregates. Before using this inhibitor, the levels of its target, PREP, were examined in SH-SY5Y cells and in human astrocytes. Since only SH-SY5Y cells expressed PREP, human astrocytes were not studied further in this context. SH-SY5Y cells were exposed to  $\alpha$ SYN-F for 24h. Thereafter the cells were washed and treated with KYP-2407 for 48h. Image analysis revealed that intracellular  $\alpha$ SYN deposits

decreased in the presence of KYP-2407 (Figure 23A-B). To rule out if increased secretion of  $\alpha$ SYN is the cause to this reduction, we analyzed the cell medium. Interestingly, the  $\alpha$ SYN levels in the cell medium were also lower in the presence of KYP-2407, proposing an increased degradation by the cells (Figure 23B). Furthermore, WB analysis showed that high molecular species of  $\alpha$ SYN aggregates were reduced in the presence of KYP-2407, confirming the image analysis results. In our earlier studies, we have shown that  $\alpha$ SYN aggregates enter the endo-lysosomal pathway. To find out whether KYP-2407 has any effect on the lysosomal pathway, we analyzed LC3BII and p62 levels, using WB analysis. Both proteins appeared to be decreased in the presence of KYP-2407 (Figure 23C). This indicates that the autophagy-lysosomal fusion and lysosomal degradation was stimulated by KYP-2407.



*Figure 23.* SY-SY5Y cells accumulate less  $\alpha$ SYN aggregates in the presence of KYP-2407 (A). Intracellular  $\alpha$ SYN, as well as extracellular  $\alpha$ SYN are reduced when cells are treated with KYP-2407 (B). LC3BII and p62 levels decline when the cells are exposed to KYP-2407, indicating increased degradation capacity. Scale bar = 10 $\mu$ m.

Calpain 1 and 2 are calcium regulating proteins and have been demonstrated to interact with  $\alpha$ SYN and increase its aggregation [122,221]. Accumulating evidence accentuates increased calcium levels in neurons and glial cells in PD [222]. Moreover, elevated calcium levels have been shown to increase  $\alpha$ SYN aggregation, which in turn disrupts calcium homeostasis inside the cell [223]. Previously, KYP-2407 has been demonstrated to regulate synthesis of IP<sub>3</sub> which is a crucial part of calcium signaling. Thus, the effect of KYP-2407 on calpain levels as well as calcium levels in SH-SY5Y cells was studied closer. Although no effect on calcium levels could be detected in the SH-SY5Y cells, KYP-2407 caused a reduction in calpain 1 and 2 levels in the presence and absence of  $\alpha$ SYN-F. In conclusion, PREP inhibition by KYP-2407 stimulated

the autophagosomal-lysosomal pathway and reduced  $\alpha$ SYN secretion from SH-SY5Y cells. PREP inhibition also reduced calpain 1 and 2 levels. However, further studies are required to find out how this reduction is beneficial to the cell.

## Future perspectives

While the papers in this thesis include several new and important findings, which contribute to the PD research field, some questions still remain unanswered and new questions have also been raised, based on the results. In the following section, ideas about how to continue the studies of glial cells in PD are presented.

Tunneling nanotubes have mainly been shown in *in vitro* models and there has been a debate whether they are only an *in vitro* phenomenon. To quantify and distinguish TNTs from other cellular protrusions *in vivo* and *in vitro*, there is a great need for specific markers that can be used to detect them. Despite many efforts, finding a more specific marker than actin-related proteins has been difficult. Recently, several studies have been able to detect TNTs *ex vivo* as well as *in vivo*, using advanced microscopy techniques, such as two photon *in vivo* imaging [174]. Nevertheless, these studies have also relied on actin staining. In order to confirm their presence and more easily study TNTs as a mechanism for disease spreading, specific detection of TNTs is essential.

Upon antigen presentation via APCs, T cells that express the specific receptor for the antigen become clonally expanded. Furthermore, T cells will express and secrete cytokines, such as IL-2, that facilitate their expansion. To further verify that astrocytes function as APCs, it would be interesting to investigate whether they cause clonal expansion of T cells. For this purpose, astrocytes can be generated from iPSCs derived from PD patients and co-cultured with T cells from the same PD patient. T cells can be generated from the same iPSCs that are used for producing astrocytes or isolated from blood. This way, astrocytes and T cells from the same patient can be co-cultured, reducing the risk for any immunological reactions. Additionally, astrocytes may be involved in T cell re-activation, meaning that the T cells have already been activated by other APCs, such as dendritic cells, before they enter the brain. In that case, T cells that travel to the brain and are re-activated by astrocytes would also clonally expand in the brain. Therefore, it would be very interesting to isolate T cells from PD mouse brains and co-culture them with mouse astrocytes that have engulfed  $\alpha$ SYN-F. Furthermore, using advanced 2-photon microscopy, one could also image T cells entering the brain parenchyma in PD mouse models and study their interaction with the stationary brain cells.

The results from **Paper III** are very intriguing and encourage us to investigate the mechanisms by which microglia and astrocytes cross-talk in PD and AD. Further experiments could be performed to elucidate exactly how astrocytes and microglia communicate with each other. For example, by treating one cell type with aggregated proteins and then adding these cells to the other cell type, it would be possible to find out which cells primarily transfer the aggregated proteins. Moreover, by inhibiting cellular contact using actin polymerization inhibitors we could study how much of the total transfer is TNT-mediated. Previous studies have shown that astrocytes and microglia can communicate through secretion of various factors [208,210,224,225]. Hence, studying the conditioned medium from the co-culture system could provide valuable insights. Further studies of *ex vivo* material from PD and AD patients can also be of interest to confirm the new findings regarding cross-talk between microglia and astrocytes.

In **Paper IV**, PREP levels could not be detected in astrocytes. However, other studies have demonstrated that microglia express PREP [226,227]. Therefore, it would be very interesting to study if KYP-2407 also has an effect on microglial degradation of  $\alpha$ SYN. Immortal cell lines, such as SH-SY5Y cells, may not be the best cell culture models for investigating a disease mechanism. Cancer cell lines have various genetic mutations, which could affect the biology of the cells. In addition, new mutations may arise due to countless number of passages. In order to confirm the pathways stimulated by KYP-2407 proposed in **Paper IV**, it would be interesting to repeat the study using primary neuronal cells derived from control cases or PD patients.

# Populärvetenskaplig sammanfattning

Neurodegenerativa sjukdomar är ett samlingsnamn för åkommor som på olika vis leder till att nervceller i hjärnan dör. Alzheimers sjukdom och Parkinsons sjukdom är de vanligaste neurodegenerativa sjukdomarna. Idag lever ungefär 30-40 miljoner människor med Alzheimers sjukdom och 6 miljoner människor med Parkinsons sjukdom runtom i världen. Dessa två sjukdomar skiljer sig åt på flera sätt men de har även en del likheter. Ålder är den största riskfaktorn för båda sjukdomarna och risken för att drabbas ökar efter 60 års ålder. Patienter som insjuknat i Alzheimers sjukdom lider av glömska och förändrad personlighet. Parkinsons sjukdom påverkar framförallt rörelseapparaten och leder till skakningar och stelhet. Dessa symptom uppstår på grund av att nervceller som ansvarar för minne och rörelse förtvinar under sjukdomarnas förlopp. Man tror att sjukdomsförloppet inleds i hjärnan minst 10 år innan symptomen visar sig. I kroppens celler produceras det kontinuerligt olika proteiner. Ibland produceras det av misstag defekta proteiner, vilka i vanliga fall omhändertas och bryts ner. Med åldern blir de mekanismer som ansvarar för omhändertagandet av dessa defekta proteiner sämre, vilket leder till ansamlingar av ”skräp” i cellerna. Följaktligen kan vissa defekta proteiner klumpa ihop sig och bilda större aggregat. Vid Alzheimers sjukdom bildas klumpar av proteinet amyloid-beta och vid Parkinsons sjukdom bildas klumpar av proteinet alfa-synuklein. Proteinaggregaten orsakar stress för nervcellerna i hjärnan och gör så att de inte längre förmår att fungera som de ska och till slut dör de. Man vet att proteinaggregaten kan spridas från ett område i hjärnan till ett annat, vilket leder till att hela hjärnan insjuknat mot sjukdomens slutskede.

Förutom nervceller finns det även andra typer av celler i hjärnan, till exempel gliaceller. De två vanligaste gliacelltyperna heter astrocyter och mikroglia. Dessa är essentiella för att nervcellerna och hjärnan ska fungera normalt. Längre trodde man att gliaceller inte fyllde någon viktig funktion hos patienter med Alzheimers sjukdom eller Parkinsons sjukdom och istället fokuserades arbetet på nervcellerna. Idag vet vi att gliacellerna tvärtom är väldigt viktiga för sjukdomsförloppet. Forskningen har visat att när nervceller dör på grund av ansamling av aggregerade proteiner så utsöndras de sjukliga proteinerna till omgivningen. Astrocyter och mikroglia, som även fungerar som hjärnans immunceller, reagerar på de sjukliga proteinerna och på de döda nervcellerna. Denna reaktion, eller aktivering, liknar den som immunceller i blodet visar som svar på en infektion av bakterier eller virus. Aktiverade astrocyter och

mikroglia börjar ”äta upp” (fagocytera) de defekta proteinerna i ett försök att bryta ner dem så att inte fler celler ska bli sjuka och dö. Gliacellerna utsöndrar även inflammatoriska signalmolekyler till omgivningen som ett rop på hjälp och rekryterar på så vis nya mikroglia och astrocyter till området. Utsöndring av dessa inflammatoriska faktorer under en längre period påverkar de närliggande cellerna negativt. Även astrocyter och mikroglia mår dåligt av att fagocytera och sekretera inflammatoriska faktorer för länge. Därför är en aktiv och effektiv inflammation viktigt för att återställa jämvikten i vävnaden. Man kan uttrycka det som att inflammationen i Parkinsonhjärnan och Alzheimerhjärnan är kronisk då sjukdomarna pågår i flera år. Syftet med den här avhandlingen var att öka förståelsen för hur mikroglia och astrocyter är delaktiga i spridningen av de sjukliga proteinerna och den kroniska inflammationen i Alzheimers och Parkinsons sjukdom. Vi har framförallt använt oss av cellkulturmodeller som genererats från humana stamceller. Därefter har vi matat astrocyterna och mikroglia med aggregerat alfa-synuklein eller amyloid-beta och studerat deras respons.

I den första studien visade vi att astrocyter tar upp stora mängder av aggregerat alfa-synuklein. Dessvärre kan de inte bryta ner det intagna materialet och istället lagrar astrocyterna det aggregerade alfa-synukleinet inuti sig. Denna ansamling leder till att astrocyterna blir stressade och börjar kontakta varandra mer. Vi kunde visa att astrocyter formar väldigt tunna rör, så kallade nanorör, för att etablera kontakt. Inuti nanorören kan aggregerade former av alfa-synuklein skickas från en astrocyt till en annan. Teorin är att astrocyterna blir överbelastade och inte kan bryta ner det aggregerade proteinet och istället distribuerar de aggregaten till varandra i ett försök att fördela bördan. Detta kan vara ett sätt på vilket sjukligt alfa-synuklein sprider sig i Parkinsonhjärnan.

I den andra studien undersökte vi om astrocyter och mikroglia kan kommunicera med T celler som är en typ av immunceller. Tidigare studier har visat att T celler som i vanliga fall inte existerar i hjärnan tar sig in i hjärnan vid Parkinsons sjukdom till följd av den kroniska inflammationen. I denna studie visade vi att ackumulering av alfa-synukleinaggregat i astrocyter leder till att astrocyterna uttrycker vissa proteiner som är oombärliga för kommunikationen med T celler. Dessa resultat fastslår att astrocyter kan prata med T celler i Parkinsonhjärnan. Fler studier behövs för att ta reda på innebörden av dessa fynd för utvecklingen av Parkinsons sjukdom.

I den tredje studien analyserade vi hur mikroglia och astrocyter kommunicerar med varandra i närvaron av aggregerat alfa-synuklein och amyloid-beta. Vi observerade att när cellerna odlades var för sig så var mikroglia-cellerna bättre än astrocyterna på att bryta ner proteinaggregaten. När astrocyter och mikroglia odlades tillsammans kunde de ta hand om proteinaggregaten på ett mer effektivt sätt. Vi iakttog cellerna närmare och påvisade att mikroglia tog

över en del av bördan från astrocyterna när de odlades tillsammans, antagligen för att de är bättre på att bryta ner aggregaten. Till exempel kunde vi detektera närvaron av alfa-synuklein i nanorör mellan astrocyter och mikroglia.

I den sista studien undersökte vi hur ett potentiellt läkemedel för Parkinsons sjukdom verkar i nervcellsliknande celler. Vi tillsatte alfa-synukleinaggregat till nervcells-liknande celler och efter att cellerna tagit upp aggregaten behandlade vi dem med ett läkemedel som heter KYP-2407. KYP-2407-behandlingen ledde till att cellens nedbrytningsmekanismer stimulerades och att alfa-synukleinaggregaten kunde rensas bort bättre. Fler studier krävs för att studera och utvärdera KYP-2407 som ett potentiellt läkemedel för Parkinsons sjukdom.

Sammanfattningsvis har avhandlingen ökat vår kunskap om hur astrocyter kan påverka spridningen av Parkinsons sjukdom och på vilket sätt astrocyter och mikroglia är inblandade i den kroniska inflammationen vid Parkinsons sjukdom och Alzheimers sjukdom. Vi har också undersökt en presumtiv medicin som skulle kunna användas för att behandla Parkinsons sjukdom i framtiden.

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نازیزانم، سیاسی گشتتان ئەکەم بۆ ئەوەی هەمیشە هاندەر و پالێشتم بوون و لە هەموو کاتێکدا گرینگیتان پێم داوه. هەروەها سیاسی تاییەتم هەیه بۆ مامۆی بەرزیم مامۆ مەسعوود که له هەموو کاتێکدا یار و پشتیوانم بوویت و ریگای تیکۆشان و هەولدانن پێم نیشان داوه بەو پروایەت گەیاندم که هیچ هەوڵ و تیکۆشانیک بیسەر نەجام نایێ. سیاسیکی تاییەتم هەیه بۆ ژنانی خانەوادەم بە گشتی و بە تاییەت دایە گەورە باوکیم دایە هاوین و دایە گەورە دایکیم دایە کۆبرا. وه نامەى خوشەویستم ژیان که بۆ من باشترین نمونهی ژنیکی هەولەر و تیکۆشەری کورد بووگە. سیاسی باوه گەورەکانم بابا هاوین و بابا چەنگیزم ئەکەم که له هەموو کاتێکدا هاندەرم بوون. له کۆتاییشدا، سیاسی ناروین کۆره پورزای نازیزم ئەکەم که هەمیشە پالێشتمی کردووه و وەک برائیکی گەورەیش ناواتی هەبوونییم هەبووه. سیاسی گ لاییکتان ئەکەم، بیگومان ئەگەر پشتگیری ئیوهی نازیز نەبوايه نەجمای ئەم کاره بۆ من قورستر و گرانتەر دەبوو.

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