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The Detrimental Duality of Microglia in Alzheimer's and Parkinson's Disease

A matter of M&Ms?

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Abstract

Neurodegenerative diseases are predicted to become a growing burden. Despite tireless research dedicated to them many gaps in knowledge remain and effective treatment options are limited. Particularly Alzheimer's and Parkinson's Disease are becoming more prevalent with an aging world population. Instead of new research into these diseases looking towards other fields may, at times, be a more time and cost effect option to discover new targets and therapeutics. In Alzheimer's and Parkinson's microglia appear to fill a dubious role seemingly able to affect disease progression both positively and negatively. Therefor this thesis will explore overlaps between Alzheimer and Parkinson pathology, with an eye on microglia, to look for targets and therapeutic options that may transition over to the other disease.

Microglia are the resident innate immune cells of the central nervous system. These cells are most comparable to macrophages and fill a wide range of tasks to support and maintain the CNS. Microglia are not one homogenous cell type and the roles microglia fill appear dependant on subtypes, with cells able to dynamically switch. The exact signals controlling the initial subtype choice and switching remain elusive with evidence suggesting, healthy and pathological, micro-environments playing an important factor. M1 microglia fill the role of inflammatory cells and M2 microglia the roles of controlling inflammation, promoting growth and survival. The effects of microglia appear to be dependent on the different subtypes present, with pathologies able to affect which subtypes of cells are present.

Alzheimer's and Parkinson's are neurodegenerative diseases that have toxic aggregates as an important part of their pathology. Alzheimer's is characterised by the formation of neurofibrillary tangles and amyloid-β aggregates. Parkinson's is characterized by the formation of Lewy Bodies and Lewy Neurites both of which consist for a large part out of α-synuclein. Microglia interact with each of these components and can contribute to accelerated disease progression.

The microglial NLRP3 inflammasomes, vesicle shuttling, and reactive oxygen species formation are involved in both Alzheimer's and Parkinson's. Each of these pathways have been approached differently by researchers studying either disease. This has resulted in different approaches for ameliorating disease models. These approaches range from utilising antibodies, genetic manipulation, cell depletion to pharmaceutical compound deployment. Any of these is one to consider translating over from Alzheimer's to Parkinson's models or vice versa which may lead to accelerate discovery of therapeutic options for either disease.

One aspect of Alzheimer's and Parkinson's research that limits translation and feels lacklustre is the inclusion of microglia subtypes. The determination of cell subtypes and effects of treatments on subtypes is often left untouched in papers. Not only could this leave out potential important findings, but as subtypes have different functions ignoring them may lead to unwarranted results and conclusions. More emphasis should be put on these subtypes in research aimed at microglia. Regardless there is clear evidence that therapeutic targets explored in other fields may be more widely applicative, some explored here but many more are sure to exist if we chose to look for them.

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Introduction

With advancements within the fields of diet and medicine in recent times life expectancies have gone up considerably. Despite this, age-related diseases are becoming more prevalent (Campbell-Taylor et al., 2014). Alzheimer's and Parkinson's in particular, diseases that harm the central nervous system, have been predicted to become an even greater burden in the future (Campbell-Taylor et al., 2014; Weuve et al., 2014). Even though tireless research and efforts are devoted to expanding our understanding and developing treatments many aspects of these diseases remain uncertain. Limiting what can currently be done to help patients.

Perhaps new insights to understand these neurodegenerative diseases can come from outside the respective fields of research. For example the discovery and development of revolutionary polymerase chain reaction arose from microbial studies observing organisms in superheated waters (Kleppe et al., 1971), likewise new insights be obtained from looking outside of the normal boundaries of research.

Alzheimer's and Parkinson's might provide a similar opportunity to learn from alternative fields, as both diseases have their pathology rooted in aggregate formation, and both severely affect the central nervous system (Muehlhauser et al., 2001; Kim & Alcalay, 2017; Venegas et al., 2017). Even though the compositions of the aggregates in question are wildly different, the management of these structures does appear to have comparable methods of interaction. Namely through resident microglia, a macrophage subtype immune cell native to the central nervous system (Bussian et al., 2018; Hansen et al., 2014; Muehlhauser et al., 2001; Pandya et al., 2017).

Therefore, the goal of this piece will be to compare the underlying mechanisms of Alzheimer's and Parkinson's as well as their individual interactions with microglia as it pertains to disease progression. This may lead to the discovery of new therapeutic targets or enhancement of disease understanding to cross over between the two diseases.

Therefore, the central question in this thesis will be: Are there points of overlap and potential new therapeutic targets or insights to be found in the fields of Alzheimer's or Parkinson's research that may be translatable to the other with a special focus on microglia? Due to the limited scope of this thesis Alzheimer's and Parkinson's will be the only diseases discussed.

To be able to answer this question, an in-depth look will need to be had regarding the following. How do microglia normally function and develop? Where do they originate from and how does the population maintain itself? What genes and processes are microglia closely involved with? What are the hallmark characteristics of Alzheimer's and Parkinson's? What is known about the development, progression and processes playing a part in these diseases? To what extend can microglia be implicated in having a role in the progression or controlling of these diseases? What aspects of microglia are involved in this? What parts of these pathways can be targeted for better control or outcome for patients? Can targets from one disease be used in the setting of the other disease?

Microglia Origin and Function

Origin and Development

Microglia are the resident immune cells of the central nervous system (Frost & Schafer, 2016; Paolicelli et al., 2011). Belonging to the innate immune system, more specifically to the macrophage family of cells, with a twist when it comes to their developmental origin. Macrophages originate from hematopoietic stem cells (Sheng, Ruedl, & Karjalainen, 2015). Microglia belong to the same family of cells and it was long thought that they also originated from hematopoietic stem cells. However, this appears to not be the case as microglia seem to originate not from the hematopoietic system but rather from yolk sac precursor cells (Alliot, Godin, & Pessac, 1999; Sheng et al., 2015). More specifically microglia appear to originate from embryonic day 7.5. Transcription factors PU.1 and IRF8 appear to play crucial roles in differentiation and migration towards the CNS or rather neuroectoderm (figure 1) (Kierdorf et al., 2013). There is evidence of microglia being one of the few, if not the only exception when it comes to macrophages having a different origin that does not have its footing in the hematopoietic system (Sheng et al., 2015; Yona et al., 2013). This different origin for microglia seems to make sense when looking at the broader picture of development. Passing a complete and functional blood-brain-barrier, which forms on embryonic day 14.5, would be a difficult task for microglia (Blanchette & Daneman, 2015; Engelhardt et al., 2003). The formation of microglia on embryonic day 7.5 thus allows microglia to populate their target environment before the BBB is fully formed which would otherwise prevent access (figure 1).

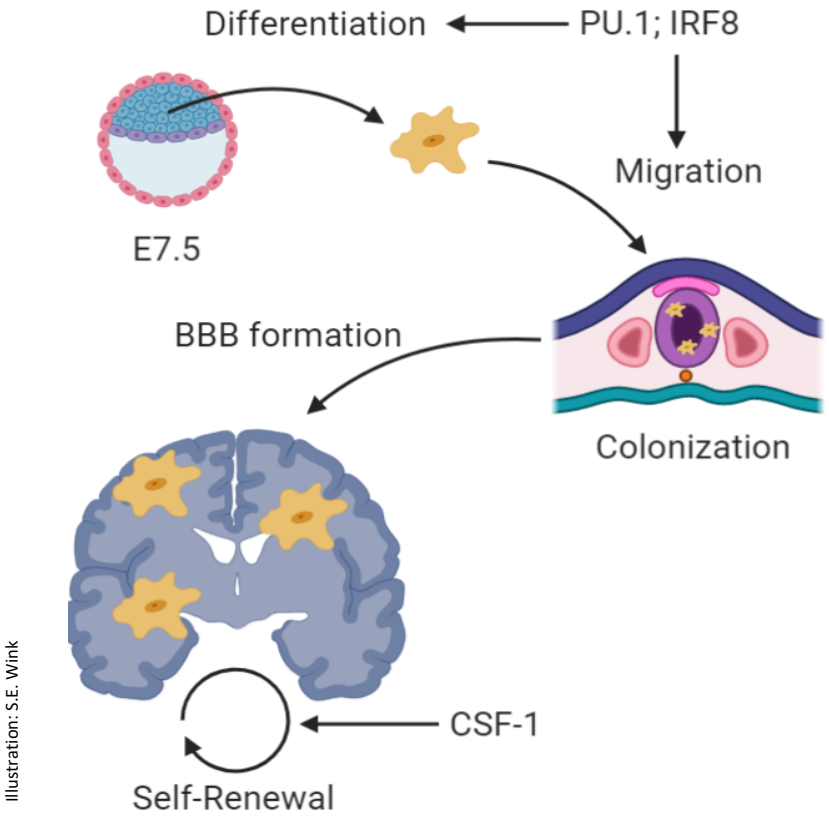

Figure 1

Microglia progenitors originate from the yolk sac on embryonic day 7.5 (E7.5). Through signaling with PU.1 and IRF8 these cells are able differentiate and migrate to the neuroectoderm before blood-brain-barrier formation. Once in the CNS the microglia population maintains itself through self-renewal controlled by CSF-1 interacting with the CSF-1 receptor on the surface of microglia.

The idea that microglia move to the CNS so early on and are then sealed in does raise another question. How does the microglia population maintain itself throughout life? Yolk sac precursors do not persist till the age of eighty years old (Yona et al., 2013). The yolk sac origin on its own does not seem to adequately support microgliosis, the expansion process of microglia in situations of disease or insult to the brain (Yona et al., 2013). This gap in knowledge made the origin of microglia in later life a compelling research topic. Studies have found both the ability of cells to move from the HSC to the brain in irradiation studies, while also that the BBB made the CNS closed off to microglia traffic (Stranahan et al., 2016). It seems to be that microglia can perform localized self-renewal, and that this is the source for new cells through life in humans (Ajami et al., 2007; Bruttger et al., 2015). This would mainly be managed through the expression of CSF-1 in the CNS environment interacting with the CSF-1 receptor on the surface of microglia (Elmore et al., 2014) (figure 1). The idea that microglia later in life could originate from the HSC and their ability to migrate across the BBB appear to be an artifact of older research methods. Seemingly irradiation performed to deplete microglia in older experiments also causes enough damage to the BBB to allow microglia to migrate across (Stranahan et al., 2016). Combining the idea that microglia originate from the yolk sac and move to the CNS before completion of the BBB and the finding that microglia are able to self-renew to maintain their population seems sufficient to explain how these cells are able to be present in the CNS and how they are able to maintain themselves through life. This allows for both casual maintenance of microglia as well as rapid population expansion in response to insult or disease targeting the CNS.

Function and Task

With the origin and developmental pathways of microglia established, it is time to examine the tasks and roles they fulfil under normal conditions within the CNS. Considering microglia are classified as macrophages the simplest starting point would be to look at their phagocytic ability.

Microglia can digest both cells and debris or assist in apoptosis management. This appears to be primarily regulated by the TREM2 pathway (Condello et al., 2018). TREM2 activation leads phosphorylation of DAP-12 causing cytoskeletal reorganization leading to the engulfment needed for some forms of phagocytosis and clearance of debris (figure 2) (Mecca, et al., 2018; Peng et al., 2010). Later, in this thesis a greater focus will be put on the potential undesirable effects of TREM2 activation. For now it is enough to assume that TREM2 mediated phagocytosis can be triggered by various receptors depending on the molecule or protein in question (Zheng et al., 2017). For example, the highly conserved toll-like receptors various cells of the innate immune system express. This will be explored in more detail regarding specific targets and interactions with Alzheimer's and Parkinson's. Their ability to phagocytose select structures is reliant on their ability to monitor their surroundings through a wide range of receptors, but also by their ability to be attracted to sites of high extracellular ATP, which can be a signal for damage (Wang et al., 2016). It is with this phagocytic ability that microglia also form a starting point for various inflammatory cascades and pathways, which can be both beneficial as well as detrimental depending on the context as will be

discussed more later. Besides a classical role of being a resident immune cell in the CNS microglia also are important players in the field of homeostasis and management of the CNS environment (Pluvinage et al., 2019). This is due to a microglia's ability to not only release key cytokines, such as IL-1β and TNF-α, that promote inflammation but also by contributing to processes involved with supporting tissues (Frost & Schafer, 2016). This support comes in various forms ranging from maintaining neurons, aiding in oligodendrocyte maturation and differentiation in the hippocampus, angiogenesis, and embryonal development of layer V cortical neurons (figure 2) (Blanchette et al., 2015; Miron et al., 2017; Zrzavy et al., 2017). Furthermore, microglia appear to play a significant role in pruning unnecessary neurons and neural connections, through the release of reactive oxygen species (ROS) and DAP12 signalling (Bar & Barak, 2019; Paolicelli et al., 2011). It must be said that the pruning of neurons mediated by microglia is not the primary way in which pruning occurs (Paolicelli et al., 2011).

This illustrates how deeply microglia are intertwined with all sorts of processes taking place within the CNS; their interaction ranging from phagocytosis, which is commonly associated with macrophage cells to supporting and managing day-to-day process and homeostasis as well as various paths in-between (figure 2). But this wide range of abilities and tasks also raises a question itself, how can one cell type fill so many distinct roles? For that a closer look at the different types of microglia that exist is necessary.

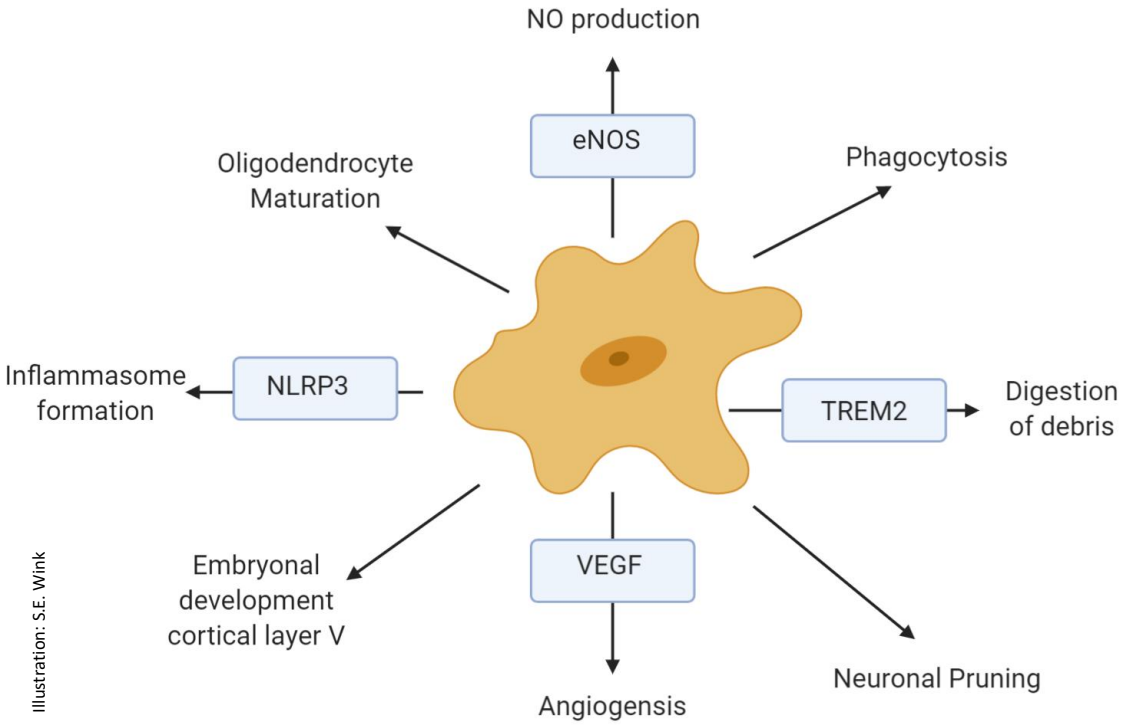

Figure 2

Microglia fill a wide range of functions within the central nervous system. Ranging from basic macrophage like tasks such as phagocytosis to influencing tissue growth, development, and repair.

M1 and M2abc subtypes

Until now, microglia have been presented as a homogenous population of cells that is the same throughout the brain, but this is not the case. Research shows that specific and functional distinct subtypes of microglia exist within the CNS classified as M1 and M2 microglia with the group of M2 microglia being further divisible into M2a, b and c (figure 3) (Orihuela et al., 2016). These distinctions are based on varying kinds of primary behaviour each of the subtypes exhibit and can be identified by different markers. Even though the different subtypes all have different niches they do appear to originate from the same cell line (Tang et al., 2016). Unlike many subtypes of cells, the distinction between M1 and M2 appears to be a dynamic and microglia can, over time, switch from one subtype to the other based on signalling factors (Choi et al., 2012). There appears to be no specific regions in the brain home exclusively to a certain subtype of microglia (Choi et al., 2012). All these points make the subtypes of microglia a complex and dynamical kind of cells that are hard to study in any setting especially in that of any pathology. Taking a closer look at all these subtypes is warranted to get a more complete picture of microglia, before discussing their role in either Alzheimer's or Parkinson's.

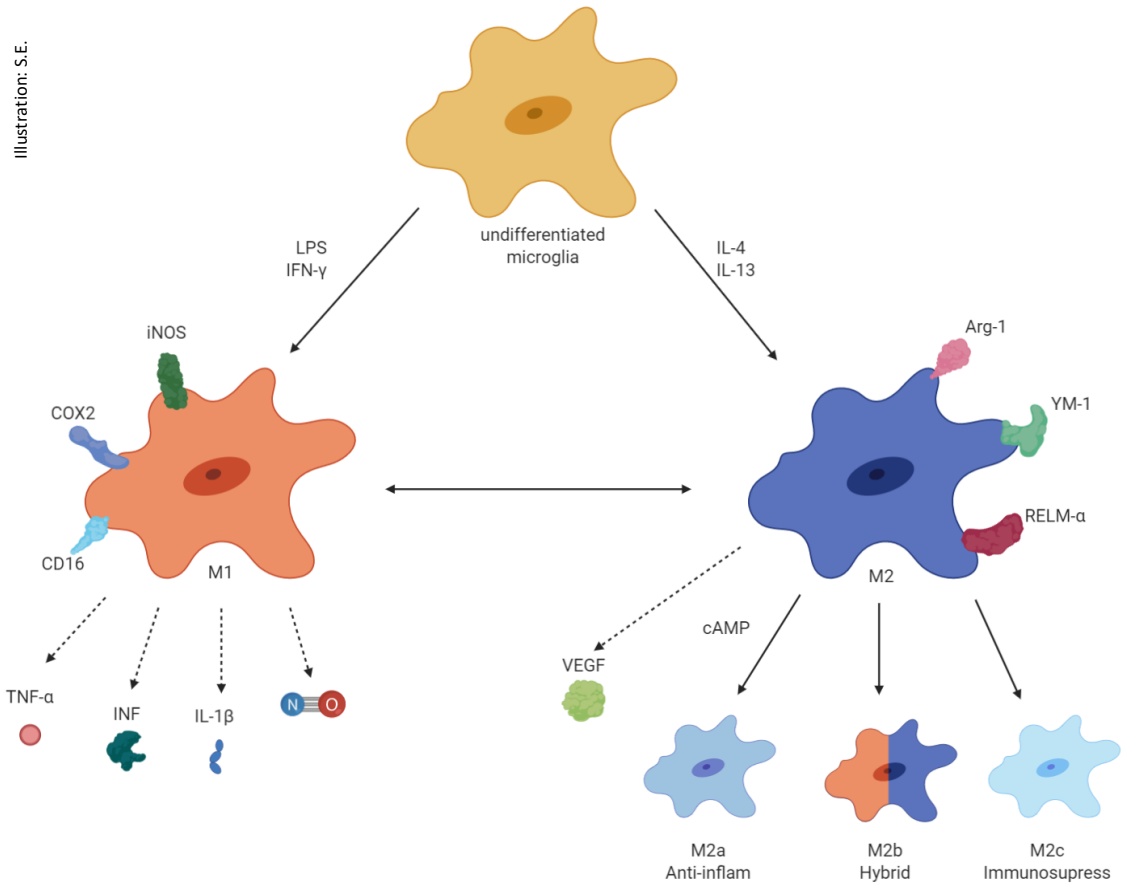

Figure 3

Microglia can appear in various forms determined by signaling molecules present in the environment they are in. Based on the type of microglia differences in functions, secretory pallet and markers are present. The different types of microglia are dynamic allowing for cells to change over time in response to what is needed.

The M1 subtype, as mentioned before, originates from the same point as M2 microglia. M1 microglia can be identified through various markers. Three of the most common ones are the expression of iNOS, COX2 and CD16 (figure 3) (Durafourt et al., 2012). In experimental settings M1 microglia are often formed using lipopolysaccharides or IFN-γ, but these signals are not always representative of normal brain chemistry signals (Durafourt et al., 2012; Fenn et al., 2012). In the setting of various diseases alternative signals can lead to the formation of M1 cells but this will be discussed more in depth later. M1's are the subtype of cells that are associated with the basic picture of microglia, with regards to their relation to macrophage, filling the role of, amongst others, phagocytosing structures, assisting in neuronal pruning, and producing a wide range of (pro)inflammatory molecules (Orihuela et al., 2016). M1 microglia have been found to produce various molecules depending on the environment they are active in, some of these products are TNF-α, INF, IL-β and a wide range of oxygen species such as ROS and NO (figure 3) (Bordt & Polster, 2014; Yang et al., 2018). Looking at the roles performed, and structures produced, M1 microglia may be considered the proinflammatory kind of microglia.

M2 microglia are mostly identified through their expression of Arginase-1, YM-1, and RELM-α (M. Ghosh et al, 2016; S. Ghosh et al., 2013; Hansen et al., 2014). For the formation of M2 microglia IL-4 and/or IL-13 have been found to be required and can also be used in research to artificially induce M2 formation (figure 3) (Sica et al., 2006; Stein et al., 1992). Some functions attributed to all M2 microglia subtypes are angiogenesis, oligodendrocyte maturation, and managing axonal (re)growth (Blanchette & Daneman, 2015; Miron et al., 2017). The signals needed for the formation of M2a, b or c cells remain uncertain. One signal that does appear to have an important role is cAMP, which, when increased, is associated with the formation of M2a microglia (figure 3) (M. Ghosh et al., 2016). M2a microglia may best be described as filling a counterbalancing role to the M1 microglia (Kalkman et al., 2017). M2a's have been found to have a strong role in anti-inflammatory signalling, whether this is through chemical signalling or through inhibition of M1 is not clearly defined (Kalkman et al., 2017). M2b microglia are described as being a compromise between M1 and M2a in terms of function, not strongly leaning towards either type (Walker et al., 2015). Lastly are the M2c microglia. These are found to fill an immunosuppressive role, but as with most microglia subtypes, few in-depth studies have truly focused on them, leaving many uncertainties to be resolved (Mecha et al., 2020; Walker et al., 2015). M2 microglia have been attributed with the production of, amongst others, VEGF, BDNF and PDGF. Though exact secretion profiles for each of the M2 subtypes remains unknown (figure 3) (Ding et al., 2018; Parkhurst et al., 2014; Su et al., 2017).

The various subtypes of microglia appear each to have a distinct and context dependant role to fill. Their ability to dynamically switch between subtypes most likely influenced by the microenvironment of the CNS they find themselves in. This repertoire of functions and the ability to adapt make microglia a worthy cell to observe in the unique settings of neurological disease, much remains to be discovered.

Alzheimer's Disease

Before the role of microglia in either Alzheimer's or Parkinson's can be evaluated the major aspects that make up the respective pathologies need to be discussed. In the case of AD this involves tau and amyloid-β.

Tau

The first of two primary components in AD is the formation of hyperphosphorylated tau constructs. The tau protein originates from an alternative splice form of the Microtubule-Associated Protein Tau, *MAPT*, gene (Spillantini et al., 1998). Under healthy conditions it is important for the stabilization of microtubules within axons thereby aiding in maintaining neuron shape and strength (Yoshiyama et al., 2007). The tau protein's functions, and activity are largely controlled by post-translational modifications, the most impactful of which is the phosphorylation (Billingsley et al., 1997; Goedert et al., 1995). Tau is also important for axonal development and trafficking. Misfolded tau-forms within neurons lead to aggregate formation in prion-like diseases commonly referred to as 'tauopathies' (Clavaguera et al., 2009). In Alzheimer's tau forms paired helical filaments with all possible isoforms forming neurofibrillary tangles (NFTs), which often are accompanied by hyperphosphorylation (Naseri et al., 2019). This hyper-phosphorylation plays an important role in the tendency of tau to form into NFTs, and one potential cause for this hyperphosphorylation is a decrease in the activity of protein phosphatase 2A, PP2A (Goedert et al., 1995). NFTs cause neuronal death through gain-of-function toxicity but also because NFTs are incapable of filling the role normal tau has to stabilise microtubules in axons (Naseri et al., 2019). Upon cell death the NFTs are released from the cells and unless processed by scavenging cells can float freely through the CNS and seed to new locations in the brain (Hopp et al., 2018). Tau pathology in AD is thought to follow a pattern of progression starting in the hippocampus spreading through the entorhinal cortex and ending in various cortical regions (Pîrşcoveanu et al., 2017). This spread seems to occur through a structurally connected pattern (Naseri et al., 2019). The fact that this spread often occurs through connected regions has sprouted the idea that this spread is mediated through cell-to-cell transmission (Gibbons et al., 2017). Where transmitted NFTs, once taken up by other neurons in a process called cross seeding, cause cell death in the new neuron starting the process over again (Andaloussi et al., 2013; Venegas et al., 2017).

Amyloid-β

The second major component of AD is the amyloid-β plaque formation. Under normal conditions amyloid function is involved with the growth and repair of neurons. Focusing on the genetics behind amyloid-β there are three genes of importance, namely *APP*, *PSEN1* and *PSEN2* (Moore et al., 2015; Musiek et al., 2015). The *APP* gene encodes the Amyloid Precursor Protein which when translated is cleaved by Presenilin 1 and 2, which are encoded in the *PSEN 1/ 2* genes (Moore et al., 2015). These proteins are part of the γ-secretase pathway and turn APP into amyloid-β (Hampel et al., 2020). The formation of amyloid-β itself does not explain

however how plaques are formed, there remains a degree of uncertainty as to what exactly triggers the initial formation of aggregates, but once formed one aggregate can, in a prion like manner, induce more aggregate formation to occur (De Strooper & Karran, 2016; Musiek et al., 2015). One way by which amyloid-β plaques can lead to cell death is through the RAGE surface receptor found on a wide range of cells throughout the body including the cells in the CNS (Cai et al., 2016; Donahue et al., 2006). Following the interaction of amyloid-β and the RAGE protein there is induction of reactive oxygen species production which causes damage to the DNA, proteins and important cellular structures leading to cell death (Donahue et al., 2006; Piras et al., 2016). As the cell death occurs amyloid-β plaques are not cleared or broken down allowing further interaction with other cells continuing the process (Donahue et al., 2006).

Alzheimer's Disease and Microglia

With a better understanding of how Alzheimer's works it is now possible to take a closer look at potential overlap between these components and microglia function to determine possible detrimental interactions.

Neurofibrillary Tangles

Firstly, looking at tau, it was already discussed how NFTs can spread along connected neuronal pathways. However, studies have also found that tau does not exist exclusively in intracellular structures but can also be identified within freely floating exosomes within the cerebrospinal fluid of the CNS (Alvarez-Erviti et al., 2011; Asai et al., 2015). Considering that apoptotic neurons can, and in the case of AD often do, contain high amounts of NFTs before collapsing it seems plausible for microglia to take in these NFTs when they clear out the debris from apoptotic neurons (Andaloussi et al., 2013). Combining this phagocytic activity with microglia their ability to produce exosomes it would be possible for exosomes containing NFTs to spread the toxic structures further and faster through the CNS than regular cell-to-cell transmission along neuronal tracks would. In fact, studies have found clear evidence of these tau filled vesicles being formed by microglia and that these contribute to the propagation of NFTs through the brain a finding strengthened by the ability of researchers to slow down the propagation of tau in their models by inhibiting the formation of exosomes (Asai et al., 2015; Fernandes et al., 2018). The ability of microglia to spread undesirable molecules that can contribute to worsening disease conditions is not unique found in AD (Heneka et al., 2018; Xia et al., 2019). Even though microglia phagocytosing tau can to some extent contribute to controlling the spread of NFTs it seems that through exosomes with NFTs more harmful due to sped up propagation (Asai et al., 2015; Bhaskar et al., 2014; Pandya et al., 2017). Besides the role in influencing tau-related neuronal problems microglia also appear to interact in various ways with amyloid-β which is thus also a topic worth discussing. Various studies have found evidence of microglia clustering around or in proximity to amyloid-β plaques. Suggesting direct interactions along various pathways between microglia and plaques existing.

Amyloid-β plaques

How would microglia interact with or get activated by amyloid-β plaques or the microenvironment surrounding them? For this there appear to be a few well observed avenues by which the interactions might occur. The first of these worth a closer look at is NACHT, - LRR, - Pyrin (PYD), Protein 3 or NLRP3 (Baroja-Mazo et al., 2014). The NLRP3 receptor is present on microglia and can detect amyloid-β in the environment surrounding microglia (figure 4) (Baroja-Mazo et al., 2014; Grottelli et al., 2019). The activation of NLRP3 leads to two distinct subsequent pathways in which the interaction between microglia and amyloid-β influences Alzheimer's (Heneka et al., 2013; Venegas et al., 2017). Both require the activation of caspase-1 for the cleaving of substrates to get going. casp-1 cleaves the normally inactive

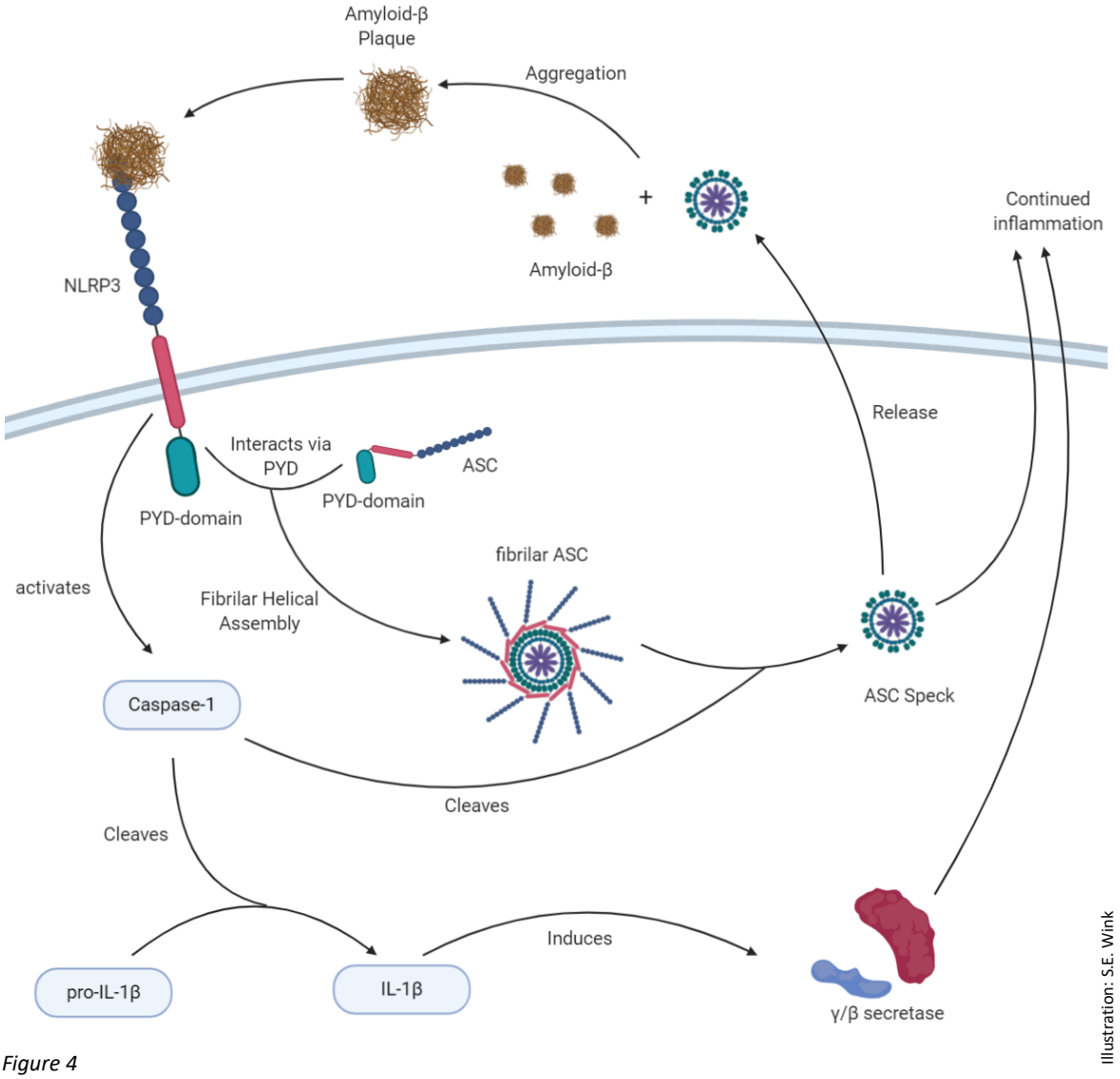

Figure 4

Amyloid-β can interact with the NLRP3 inflammasome expressed by microglia. After activation of the NLRP3 inflammasome casp-1 is activated and cleaves pro-IL-1β into an active form leading to subsequent release of secretases and continued inflammation signaling. Upon this interaction the PYD of the NLRP3 interacts with the PYD domain of ASC to lead to ASC fibrillar helical assembly. This is then cleaved by the early activated casp-1 forming ASC specks. When released these specks not only contribute to the continuation of the inflammation but also are able to interact with amyloid-β causing additional plaque formation.

form of IL-1β, pro-IL-1β, to its active form (figure 4) (Chan et al., 2019). The active form of IL-1β can itself promote the maintenance of inflammation but also induce the production of γ and β secretases which, once secreted by microglia, leads to the continuation of the inflammation (figure 4) (Feng et al., 2019). The increase in released amyloid-β can again interact with the NLRP3 inflammasome of microglia thereby resulting in a positive feedback loop.

The second way in which NLRP3 is involved with amyloid-β is through the formation and recruitment of the adaptor protein apoptosis-associated speck-like protein, ASC (Nuvolone et al., 2015; Venegas et al., 2017). The ASC interacts with the NLRP3 complex through a shared PYD domain on both structures. This interaction leads to ASC helical fibrillary assembly. The newly formed structure can then, by recruiting the earlier activated casp-1, be cleaved into ASC specs (figure 4) (Venegas et al., 2017). Following their formation, these specs are released from microglia, and they themselves appear to fill two functions as well. When these specs are taken up by surrounding (microglia) cells they can promote the maintenance of the immune response keeping the immune system active and possibly exacerbating conditions over time (Baroja-Mazo et al., 2014). Another effect and arguably more significant is the ability of the ASC specs to directly interact with amyloid-β floating in the CSF (figure 4) (Venegas et al., 2017). This interaction can then lead to further aggregation and speed up the rate at which aggregation occurs. Considering this leads to more amyloid-plaque formation it will lead to increased interactions with the NLRP3 of microglia that in turn will produce more ASC forming a positive feedback loop (figure 4).

The second major interaction between microglia and amyloid-β that can potentially adversely affect AD pathology is the Triggering Receptor Expressed on Myeloid cells 2, or TREM2 (Benitez et al., 2014). TREM 2 is a V type IG receptor, which in the brain, is exclusively expressed by immune cells (Colonna etal., 2016). Despite the exact ligands for TREM2 remain elusive, however studies have been able to find the effects on microglia-AD interaction (Benitez et al., 2014). Functions associated with TREM2 are the supposed inhibitory effects it can exert over inflammatory signalling and promote cell survival (Ulland et al., 2017; Zheng et al., 2017). A different and more established role of TREM2 is its role in regulating phagocytosis (Hsieh et al., 2009; Schoepp et al., 2017). This effect is also reflected in the relationship between plaque associated microglia and TREM2 levels. Microglia that crowd around these plaques show increased expression levels of TREM2 (Jay et al., 2017). The question remains however whether the increase in TREM2 leads to the clustering or whether the presence of amyloid-β leads to the upregulation of TREM2 expression by microglia. Researchers experimenting with TREM2 deficient models have shown reductions in the amount of microglia clustering around plaques (Jay et al., 2017; Ulrich et al., 2014). Considering however that TREM2 also is involved in partial cell survival signalling it could be that the TREM2 deficient models have reduced plaque association due to an overall lowered total count of microglia as more cell death occurs (Poliani et al., 2015). Whether TREM2 leads to clustering or amyloid-β leads to upregulation remains to be seen by further research.

A different discovery from the TREM2-deficient studies was that in early AD models lacking TREM2 there appeared to be a reduced plaque count and reduction in the area affected by amyloid-β (Condello et al., 2018; Jay et al., 2017; Ulland et al., 2017). While in late stage AD models this result was flipped and TREM2 deficiency was associated with increased plaque size (Chakrabarty et al., 2016; Gratuze et al., 2018). An explanation for this could be that due to lowered phagocytosis and subsequent internal interactions with NLRP3 the microglia lacking TREM2 had reduced expression of ASC specs and thus less cross seeding leading to slowed progression. Whereas TREM2 deficiency in late stage AD plaques were not correctly managed without phagocytosis because of limited to no interaction with microglia lead to worse conditions. Sadly, assessment of ASC specs was not part of any of the studies, so this remains speculation. Irrespective of the role NLRP3 ASC specs might have played a role in importance of TREM2 is illustrated here as AD progresses. Alternatively, to explain TREM2 deficit results through NLRP3 lies with the inability of microglia to adequately cluster around plaques. In early stages this may not be harmful but in late stages neglection of proper aggregate management may lead to worsening of conditions. Further studies will need to assess the exact inner workings of this TREM2 deficient model, but it has already been shown that TREM2 is a player in AD with varying significance dependant on time and place.

Illustration: S.E. Wink

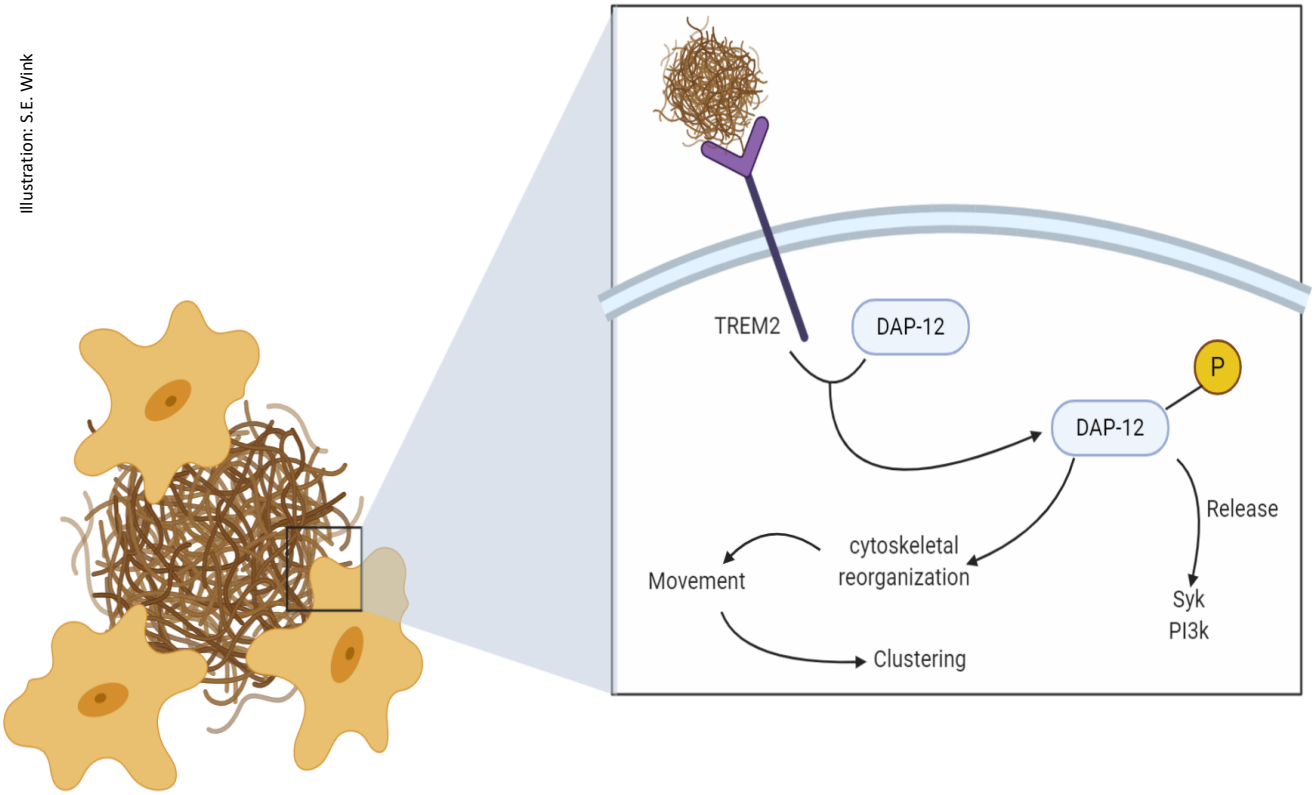

Figure 5

Microglia clustering around amyloid-β leads to internal signaling via the TREM2 receptor. Leading to the phosphorylation of DAP-12, which in turn leads to movement of the cell allowing for clustering but also further downstream signaling via Syk and PI3k.

The exact ligands for the TREM2 receptor are still a topic of research. The effects on cellular processes once TREM2 is activated have seen more conclusive observations. Once activated, TREM2 can induce DAP12 phosphorylation, leading to cytoskeletal reorganization enabling cellular movement for clustering or phagocytosis. Phosphorylated DAP12 is also able to recruit Spleen tyrosine kinase, or Syk (Mecca et al., 2018). Downstream targets of Syk are PI3k, MAPK and influx of Ca2+ thereby influencing a wide range of further processes (Wang et al., 2016). PI3K can influence the PI3K/AKT/mTOR pathway (Peng et al., 2010). MAPK influences the ERK1/2 signalling route important for synaptic plasticity, apoptosis, and senescence regulation (Klegeris et al., 2008). Combined these pathways show various processes involved in controlling microglia behaviour. The chronic activation of microglia along the TREM2 pathway may also lead to increased production of reactive oxygen species further contributing to neurodegeneration. Despite many facets of the TREM2 receptor and its influence remaining unknown it is apparent that this receptor can in fact play an important role in microglia behaviour in pathological settings, suggesting that this is part of microglia worth further investigation.

Alzheimer's Disease and Microglia subtypes

There appears to be supportive evidence linking NLRP3 and TREM2 signalling pathways in microglia to AD. This link implicates microglia in the process of slowing down AD progression, through phagocytising aggregates but also, and more interesting, the role of microglia in tau propagation through exosomes and increasing overall amyloid-β plaque burden through cross seeding. There are however still plenty of gaps in knowledge to fill. Studies observing microglia interactions with singular components of AD are quite common, though more emphasis could be put on the effects of microglia on both tau and amyloid-β at the same time.

A different aspect to the studies focussing on AD models is that they often lack do not pay attention to the distinction between microglia subtypes and frequently do not include this as part of their research. Taking these subtypes into account could well improve result clarity. Some microglia subtypes have opposing functions and thus confounding factors potentially present cannot be excluded. Without researchers explicitly going through the nature of their microglia populations deductions are the only option to assess the influence of potential differences.

Using the information from the microglia subtypes combined with the studies on microglia AD interaction the following can be reasoned. Central in both the NFTs management and the amyloid-β aggregation appears to be a role of microglia. Irrespective of whether this process is supposed to have been developed to clear these structures, evidence suggests that management of these structures can cause worsening of the AD pathology. The phagocytic, exosome and inflammasome nature of microglia can be largely attributed to microglia of the M1 subtype. Meaning that these cells could play the major role in the problems caused by microglia in Alzheimer's.

Unfortunately, without studies explicitly adding a focus on microglia subtypes in their studies logical reasoning will be the end point, unsupported by clear findings and results leaving

potentially important gaps in knowledge. Therefore, additional attention should be put on incorporating microglia subtypes as part of future studies. Various options could be explored to accomplish this. For example, through better defined markers and culture protocols more control could be had over the types of cells found in models. Shedding more light on the various factors during different stages of AD could allow for insights into the balance between M1 and M2 microglia type switching. Single cell sequencing may aid in this to determine the exact breakdown of microglia subtypes in various locations and stages in AD could be achieved. The method researchers could use would vary but including more attention to these subtypes. This could enhance result quality as more data noise could be excluded while also allowing for insights into new correlations.

Alzheimer's Disease therapeutic targets related to Microglia

With some of the major contributing pathways between AD and microglia discussed it is now possible to try and come up with approaches to improve the dynamic between the two to slow down microglia mediated Alzheimer's progression. As discussed earlier various pathways within microglia can potentially lead to accelerated AD progression. This would thus also mean that various potential targets are up for being considered.

One of the possible approaches that has been explored and is worthy of further consideration would be specifically aimed at tau propagation through the exosome formation. An option that has been explored by researchers is 'ceramide' the synthesis of which relies on sphingosinemylinase-2, nSMase-2 or Smpd3 (Asai et al., 2015). This enzyme has been shown to be inhabitable through both iRNA methods but also through pharmacological targeting via GW4869 (Asai et al., 2015). This blocks the formation of the tau filled exosomes by preventing exosome formation and could slow down the cell-to-cell transmission made possible by microglia that occurs under normal conditions (Asai et al., 2015). These kinds of compounds could and should be further explored, as more options often leads to more nuanced end results that can be applied to specific conditions and could lead to useably therapeutics to limit exosome mediated transmission.

Tau related approaches are only one aspect of AD that is open for targeting, and only of limited potential because of a reliance on timely targeting. Tau pathology is considered primarily important in early AD development, often before diagnosable symptoms may arise, this would mean that targeting tau would be reliant on early screens. This reliance would leave considerable opportunity for patients to slip through screens and then being too far along for tau targeting to be of any significant use.

On the other hand, for late stages of AD, an alternative option is to try and target amyloid-β microglia interactions. Targetting of amyloid-β microglia interaction would primarily be in the NLRP3-inflammasome and its downstream targets. NLRP3 contributes to cross seeding with amyloid-β through ASC-specs. Targetting this inflammasome could aid in slowing down the aggregate formation. One approach would be to target the ASC specs themselves with antibodies preventing them from interacting with plaques in the first place (Venegas et al., 2017). A more drastic approach would be to interfere with the ability of NLRP3 to interact

with ASC, which occurs through their mutual PYD domains. This would run into two potential walls however: one is that this interaction occurs inside of the microglia meaning that not only would drugs or therapeutics have to be carried across, the already hard to pass, blood brain barrier the second wall would be for drugs to make it into the targeted cell type. An alternative is to adopt genetic modifications through gene editing tools outside of a patient and reintroduce microglia into the CNS. Though this might be even trickier than the targeting of the PYD domain through compounds as controlling genetic editing to such an extend is still difficult, with plenty of off-target effects for this to be an effective strategy. This limitation would be on top of the already existing risk of unforeseen side-effects that might occur, which when caused by genetic changes, would be near impossible to revert compared to stopping a drug therapy.

TREM2 is another pathway explored with regards to its role in Alzheimer's progression, but this does not appear like much of a viable target to me. TREM2-deficient models may show an advantage in early AD. This was however not the case of late AD (Jay et al., 2017; Krasemann et al., 2017; Ulland et al., 2017). This time dependant distinction might make this approach too complex to adequately manage compared to alternatives. Not only would one need to account for methods of function, delivery and side effects but also be able to make a clear distinction in when the switching point occurs in TREM2's importance for AD progression. These complications do raise the question if research efforts would not be better spent elsewhere.

A final way in which microglia AD interactions could be slowed down would be through simply depleting the microglia population within the CNS potentially through deploying CSF-1R inhibitors (Renee et al., 2015). This would expose a patient to external threats as the innate immune system would not be operating at full strength. Considering there would still be other parts of the immune system left to protect the CNS, even in a diminished capacity, the trade-off for potentially extending memory and cognitive capabilities might be worth it to some. AD often affects the elderly, influencing the immune system could lead to the choice between quality of years versus quantity of years.

Going over the various AD microglial interactions there appear to be various potential points of intervention, with many more surely to be uncovered over time, some more drastic, over the top and risky than others. The limited knowledge regarding microglia subtypes and their role in AD does impede planning ahead and exploring therapeutic avenues. Rebalancing microglia population built up or deploying specific microglia subtypes may be an alternative right now the only possibility would seem targeting pathways. For this reason, more emphasis should be placed on studying these subtypes as this can go a long way in discovering new options, regardless of this there do seem alternative pathways that are worth exploring until then.

Parkinson's Disease

A-Synuclein

Comparable to Alzheimer's, Parkinson's is another neurodegenerative disease that has a strong link to aggregating proteins in its pathology. Whereas aggregates in AD consist of amyloid-β in Parkinson the main aggregating protein is α-synuclein (Spillantini et al., 1998). This makes Parkinson a member of the synucleinopathies, as is AD a tauopathy. Parkinson's is characterized by the loss of dopaminergic neurons, neurons that produce dopamine, in the substantia nigra pars compacta located in the midbrain (Lees et al., 2009; Spillafntini., 1997). The substantia nigra pars compacta is also the location that happens to contain the highest density of microglia in the CNS (Kim et al., 2000; Lawson et al., 1990). The loss of these neurons and other symptoms of PD have their roots in the formation of so-called Lewy Bodies and Lewy Neurites, these structures consists for a majority of α-synuclein aggregates (Spillantini et al., 1997). The progression of PD strongly correlates with the progression of the Lewy Body locations much in the way cell-to-cell transmission occurs with tau in AD (Cheng et al., 2018).

While α-synuclein's exact normal biological function remains somewhat uncertain, it has been associated with transport vesicle biogenesis and dynamics (Spillantini et al., 1998). α-Synuclein lies encoded in the *SNCA* gene and point mutations, duplications and triplications of this gene are risk factors for developing PD and can be traced back to the formation of familial disease (Devine et al., 2011; Zarranz et al., 2004). Translated α-synuclein consists of three main regions within the structure, an amphipathic region, a non-amyloid-β component or NAC and an acidic tail (Giasson et al., 2001). The ability of α-synuclein to assemble into various undesirable configurations such as aggregates is reliant upon posttranslational modifications such as phosphorylation of serine 129 and causes the structures to develop a gain-of-function toxicity (Fujiwara et al., 2002). The pathological nature of α-synuclein is not limited to one form of aggregates, but also oligomeric and fibrillar aggregates can cause toxic reactions and activate various pathways upon interacting with the right substrates (Lees et al., 2009; Spillantini et al., 1997; Zarranz et al., 2004). The kind of modifications are dependent on the local activity of other cells and proteins but overtime lead to the formation of the Lewy Bodies and Neurites (Spillantini et al., 1998).

More interesting is the evidence suggesting that microglia interact with α-synuclein and its various aggregated forms and can internalize these structures through phagocytosis (Stefanova et al., 2011). The exact method by which the phagocytosis occurs is not completely certain, but studies suggest that the highly conserved toll-like receptors play an important part in this process, along with clathrin dependant pathways and even Fcγ or scavenger receptors (Cao et al., 2012; Choi et al., 2012). Upon phagocytosis of α-synuclein a wide range of signalling routes can be activated, some of which advance progression of α-synuclein within the CNS, much like how microglia can spread of tau through exosomes or amyloid-β cross seeding as discussed earlier (George et al., 2019). Following phagocytosis α-synuclein have

been found to, like tau, get packaged into exosomes and through release of these vesicles microglia appear to lend a helping hand in the cell-to-cell progression of PD (George et al., 2019; Xia et al., 2019). Comparable to the studies observing exosomes that facilitate tau propagation researchers observing the role of exosomes in PD had comparable results. The exosomes formed by microglia were found to contain the protein of interest, either tau or α-synuclein. Additionally, introducing these exosomes into models would lead, in both AD and PD models, to propagation of disease associated complications (Asai et al., 2015; Xia et al., 2019). Inhibition of the formation or microglia activity with regards to exosome formation lead in both kinds of studies to reductions in disease progression. Contributing to this is the suggested ability of these filled exosomes to inhibit autophagy of the filled vesicles limiting the clearance of α-synuclein via the AKT/mTOR pathway (Xia et al., 2019). Specifically, contributions to PD occur as microglia with α-synuclein vesicles have been found to be moving towards the substantia nigra and the dopaminergic neurons located within (George et al., 2019; Xia et al., 2019). The phagocytosis of α-synuclein and subsequent cell-to-cell transmission by microglia is but half the story when it comes to PD.

Parkinson's Disease and Microglia

There is another range of pathways that get activated within microglia by α-synuclein that appear to play a role specifically in PD development and progression. Considering how PD like AD has a strong aggregate component in its pathology it seems worthwhile to first explore comparable pathways between PD and AD and see if there is overlap to be found before looking at unique aspects in PD- microglia interaction.

Studies have found that artificial activation of microglia through lipopolysaccharides can lead to accelerated degeneration of dopaminergic neurons in the substantia nigra pars compacta (Zhang et al., 2005). One pathway that can be activated through this method is the earlier discussed NLRP3 inflammasome pathway (Panicker et al., 2019). The interaction between PD and NLRP3 appears to go along a comparable track as the interaction between AD and NLRP3 (Chatterjee et al., 2020; Venegas et al., 2017). Fibrillar α-synuclein aggregates, through activation of the toll-like receptor 4 triggers the activation of MAPK leading to NF-kB activation (figure 6) (Klegeris et al., 2008; Stefanova et al., 2011). Following activation NF-kB is translocated to the nucleus and interacts with the DNA of the microglia. The NLRP3 inflammasome is then formed and activated resulting in the release of IL-1β able to induce cell death which may cause further release of α-synuclein to create a positive feedback loop (figure 6) (Klegeris et al., 2008). The activation of the NLRP3 inflammasome also promotes the release of ASC specs which are, as with AD, able to propagate NLRP3 inflammasome (Han et al., 2019; Panicker et al., 2019). The activation of NLRP3 through α-synuclein can cause the production and release of casp-1 (Heneka et al., 2013; Nuvolone et al., 2015). The release of this casp-1 on its own may be the least consequential result of the activation of NLRP3 in PD so far but upon closer examination this release causes further downstream problems. Fibrillar α-synuclein can activate the NLRP3 inflammasome only through the toll-like receptor 4 path, both fibrillar and monomeric α-synuclein aggregates can interact with and activate the toll-like receptor 2 (figure 6). This activation of the TLR2 then leads to the production and release

of pro-IL-1β (Reed-Geaghan et al., 2009). This form requires activation through cleaving before it can do anything but that is where the earlier released casp-1 comes back into the picture. The casp-1 cleaves the pro-IL-1β into active IL-1β. Thus, the various forms of α-synuclein aggregates can trigger different pathways on microglia which all in their own way, and through interlinking points, lead to the release of cytokines. These cytokines can contribute to further aggregate release but also have the ability to maintain the inflammatory reaction taking place through ASC specs (Gordon et al., 2019; Han et al., 2019). Combined this suggests that, as with AD, in PD the NLRP3 inflammasome might be a target for the development therapeutics for.

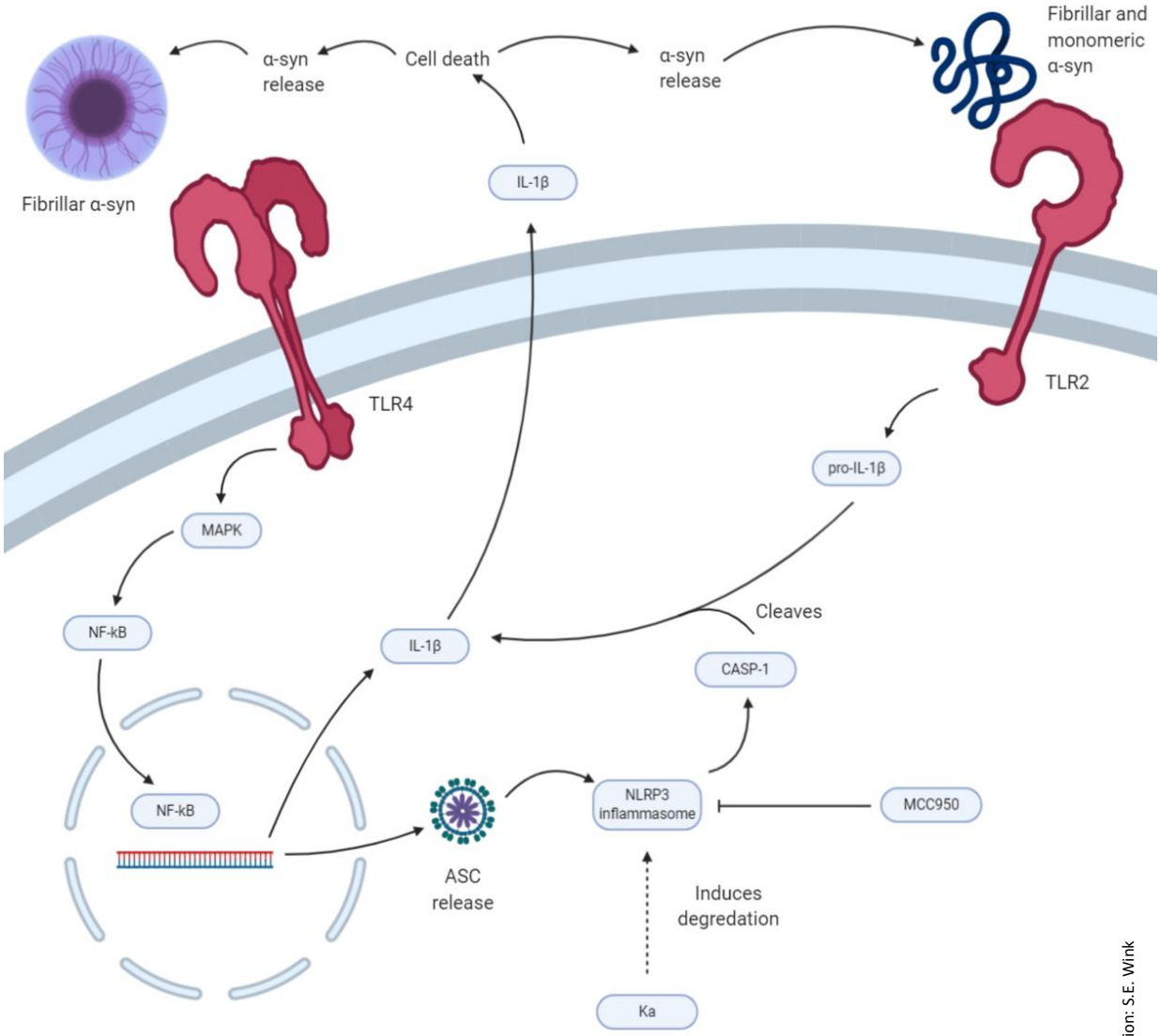

Illustration: S.E. Wink

Figure 6

Fibrillar α-synuclein can interact with TLR4 to activate MAPK, which in turn, leads to NF-kB activation and movement to the nucleus. This then leads to the transcription of IL-1ϐ and ASC. ASC then leads to the formation of the NLRP3 inflammasome and activation of casp-1. Fibrillar and monomeric α-synuclein can interact with TLR2 to promote the formation of pro-IL-1ϐ which can be cleaved by the casp-1 into IL-1ϐ. Il-1ϐ once released can lead to cell death triggering more α-synuclein forms to be released.

The TREM2 pathway in PD, unlike with AD, seems to be less of a target to consider. Given that TREM2 in AD has an apparent duality to it with a time dependent positive or negative influence of disease progression (Jay et al., 2015). This does not seem to be the case for TREM2 in PD, where it appears that models lacking TREM2 are, regardless of timing, less viable (Guo et al., 2019). In PD does it also not seem that promoting the TREM2 pathway yields any significant results for PD models (Wilson et al., 2020). Suggesting that unlike the NLRP3 pathway TREM2 may not be a target that should be highly prioritized in research for PD progression.

This said TREM2 and NLRP3 may not be the two most central components in α-synuclein mediated problems in PD. A potentially much more damaging cause from the interaction between α-synuclein and microglia could be through increased production of reactive oxygen

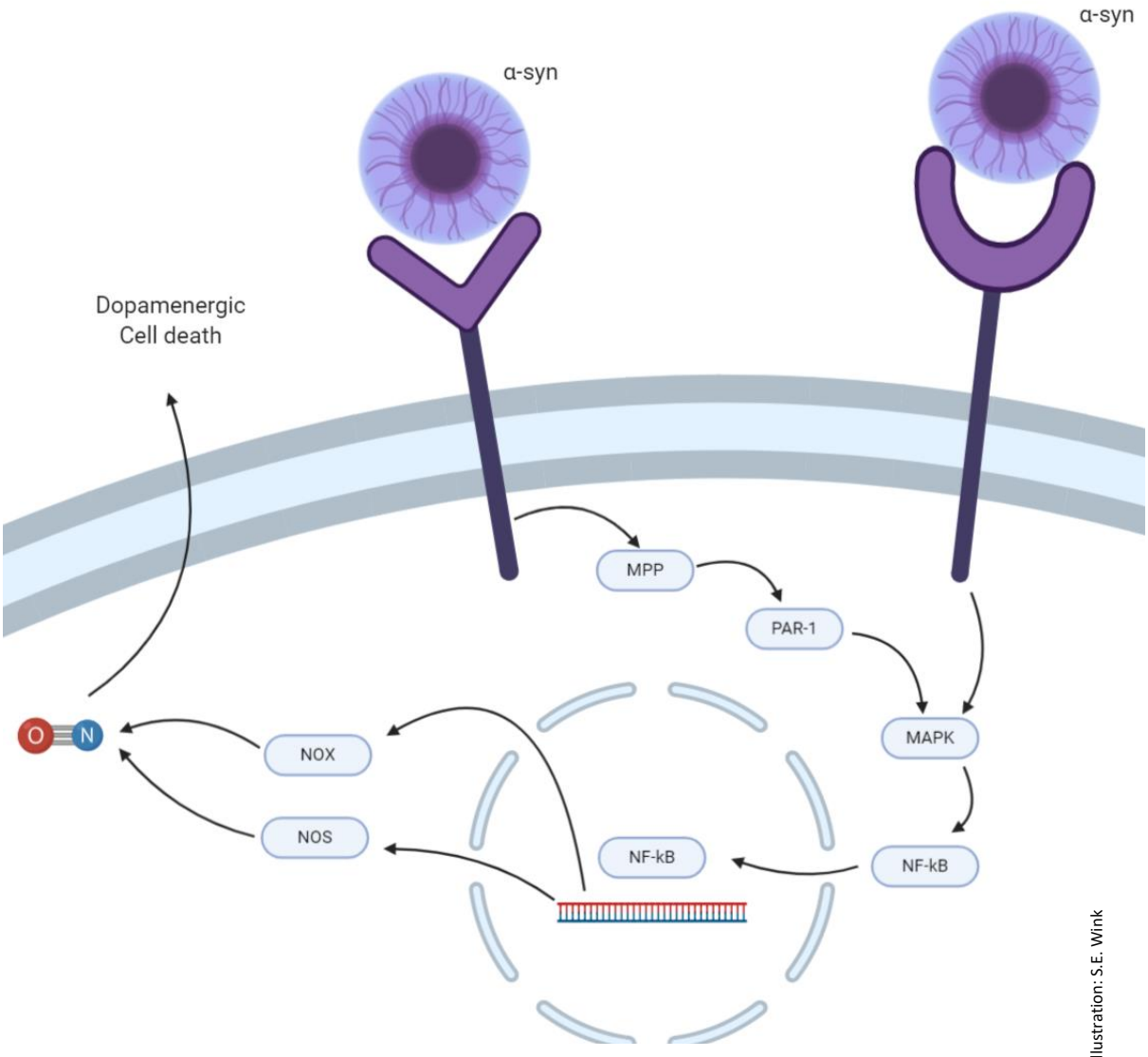

Figure 7

Through interacting with an unknown receptor α-synuclein can trigger the activation of MAPK. MAPK then leads to activation of NF-kB which upon interaction with the nucleus can trigger the transcription of NOX and NOS. NOX and NOS then can produce NO which when released into the CNS is able to cause dopaminergic cell death. Microglia interacting with α-synuclein may also lead to the formation of MPP. MPP then activates the production and release of PAR-1 which can then trigger in an auto or paracrine manner with microglia leading to the formation of NO.

species, nitric oxides, and superoxides (Gao et al., 2002). Lipopolysaccharide mediated activation leads to increased production of these molecules from microglia and α-synuclein has been found to show comparable effects in suggesting that this may be a worthwhile target (Gao et al., 2002). Add to this the fact that dopaminergic neurons are particularly susceptible to cell death triggered by oxygen species and their proximity to high counts of microglia it seems reasonable that part of the cell deaths can be attributed to microglia that produce oxides (Le et al., 2001; Shavali et al., 2006). Further supporting this is the effect of superoxide dismutase and L-NIL, both inhibiting the availability or production of different oxygen species leading to reduced dopaminergic cell death in the presence of activated microglia (Le et al., 2001). This raises the question which processes are involved in the production of these molecules and can they be targeted?

With regards to pathways involved in the generation of oxygen species a couple of candidates stand out. When interacting with α-synuclein besides the TREM2 and NLRP3 a wide range of smaller but arguably more impactful routes are set in motion. α-Synuclein causes the activation of NF-kB upon interacting with microglia. This interaction does not only activate the NLRP3 inflammasome but also leads to the generation of NOX and NOS2 both of which can induce the production of NO by microglia (figure 7) (Gao et al., 2002; Tang et al., 2014). The production of oxygen species through α-synuclein is not limited to NF-kB. ERK1/2 and p38 MAPK can be activated by microglia interacting with α-synuclein and these may lead to the production of reactive oxygen species (Klegeris et al., 2008). Another way to produce oxygen species is through the generation of Matrix Metalloproteinase, a zinc dependant endopeptidase, or MPP, upon interaction between microglia and α-synuclein. Once formed MPP is secreted by microglia, after which the molecule can interact in an autocrine and paracrine manner with other microglia to produce Protease Activated Receptor 1, or PAR-1. PAR-1 can regulate NF-kB and MAPK and is thus indirectly able to promote production of NO; ROS and even to promote proinflammatory signalling (figure 7) (Sanchez-Guajardo et al., 2015). The produced NO, ROS and other oxygen species when released in the substantia nigra pars compacta can lead to dopaminergic neuronal cell death. Unfortunately, the exact way PAR-1 activation is through MPP is still not completely figured out (Sanchez-Guajardo et al., 2015).

Together these studies show how through various means of interacting with microglia α-synuclein can lead to worsening of PD. Through expanding and facilitating further spread of aggregates through exosomes, through the release from dying neurons or through inducing neuron death. By producing a range of oxygen species that are toxic to the sensitive dopaminergic neurons in the substantia nigra microglia appear to play a significant role in PD.

Parkinson's Disease and Microglia subtypes

The production of (pro)inflammatory signals such as IL-1β and the generation of molecules such as ROS and NO is a characteristic attributed to the M1 subtype of microglia. Considering the various interactions α-synuclein has with microglia that help towards the progression and pathology of PD it seems that M1 microglia are the main culprit here. This then raises the question of how M1 subtypes are either generated or maintained in microglial populations in PD.

One plausible mechanism that appears to manage M1/M2 microglia subtypes is the H3K27me3 demethylase Jumanji Domain containing 3, or Jmjd3. Inhibition of this protein, via knockdown, has been shown to increase counts of M1 microglia and lead to elevated neuronal death (Tang et al., 2014). This appears to be due to the inhibition of Jmjd3 leading to elevated iNOS and IL-1β (figure 8) (Tang et al., 2014). Knockdowns of this protein lead to

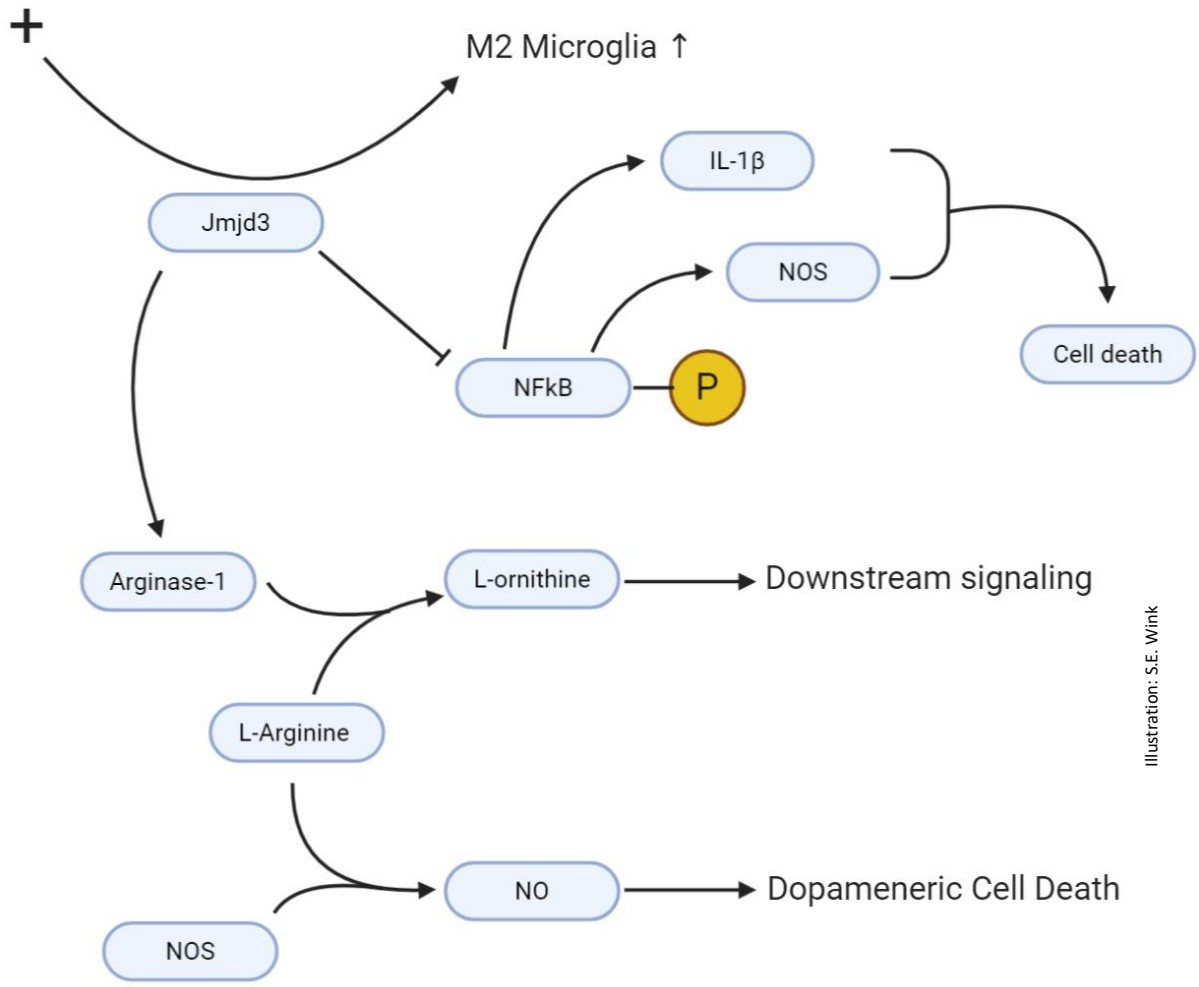

Figure 8

Jmjd3 inhibits NF-kB phosphorylation thereby indirectly inhibiting the formation of IL-1ß and NOS and thus can prevent cell death. Jmjd3 is also able to promote the activity of arginase-1 which competes with NOS for L-arginine. Through this competition less L-arginine is converted into NO and instead into L-ornithine. Promoting the activity of Jmjd3 has been done through stimulation of microglia with IL-4 and through viral inductions of gain-of-function approaches.

significant increases in NF-kB phosphorylation, which itself partly explains the increase in pro-inflammatory signalling (Tang et al., 2014). A downstream target of Jmjd3, Arginase-1, competes with iNOS for a shared substrate, L-arginine, which iNOS converts into NO. This means that when Jmjd3 is inhibited Arginase-1 is no longer activated and thus allows for more of the available L-arginine to be converted by NOS into NO (figure 8) (Tang et al., 2014). Not only is Jmjd3 involved in microglia subtype control, but also its downstream effects can directly modulate NO and proinflammatory signal production. The exact way that α-synuclein and Jmjd3 interact with each other remains to be further investigated.

With some of the various interacting pathways observed it seems that, as with AD, microglia appear to not only fill a positive role within the setting of PD. While attempting to control α-synuclein aggregates, microglia more often seem to be agents in exacerbating PD, be it through supporting the spread of α-synuclein, by producing high quantities of pro-inflammatory signalling molecules or even by contributing to dopaminergic cell death through the production of oxygen species. This means that potential therapeutic targets for PD exist within or in the pathways involved with microglia. In addition to this the example of Jmjd3 and it seems to be that microglia subtypes could be a future research topic for PD.

Parkinson's Disease therapeutic targets related to Microglia

When considering the aspects that lead to PD progression, there are a couple of main processes that come to mind as being potential targets. These would be the generation of inflammatory feedback loops (such as with NLRP3), which lead to an endless vicious cycle of signalling leading to neuronal degeneration or the formation of reactive oxygen species that are associated with dopaminergic neuronal cell death. Lastly, the subtype identities of resident microglia contributing to this all could be an interesting target.

Looking at the NLRP3 path for manipulating microglia there have been a few options suggested. One option researchers have explored is the use of a small molecule called kaempferol, or Ka (Han et al., 2019). Ka is a natural polyphenolic small molecule found in various dietary sources (Han et al., 2019). When used in PD models, it has shown marked reduction in neurodegeneration. This reduction was not only attributed to the reduced NLRP3 expression, but also to select autophagy of NLRP3 inflammasome material (Han et al., 2019). This autophagy could be inhibited through 3-methyladine, suggesting the NLRP3 autophagy degradation was taking place through the ubiquitin-auto-lysosomal pathway (Han et al., 2019). The use of Ka can thus possibly be used in preventing, and even halting, the long-lasting self-sustaining inflammation mediated by microglia and neuron damage that is caused by the associated release of IL-1β (figure 6). Another way of potentially targeting the NLRP3 inflammasome that researchers have explored is through a pharmaceutical compound called MCC950 (figure 6) (Dempsey et al., 2017). This drug leads to blockage of NLRP3 and prevents further signalling from taking place. A third option regarding NLRP3 could be comparable to the method proposed for preventing cross seeding of amyloid-β by microglia in AD. Where antibodies directed towards ASC specks could block their ability to promote the sustaining of the α-synuclein-NLRP3 triggered inflammation (Venegas et al., 2017). This however has not been an objective of a study as of now, so the use of these antibodies in the setting of PD is

purely speculation based on alternative use studies. These are just a few examples that have the potential of being developed into therapeutic options in the future.

Besides targetting NLRP3, another option could be to target either the generation or presence of oxygen species as they contribute heavily to dopaminergic cell death. A rather brute force approach to this could be through simply seeing whether administration of superoxide dismutase or iNOS inhibitors (such as L-NAME) can lead to any significant results when it comes to neuronal death in the substantia nigra par compacta. These options could possibly run into the problem of administration as well as possible side effects in the cardiovascular system, iNOS inhibitors, as NO is an important vasodilator (Csanyi et al., 2006). Regardless of this L-NAME may still provide insights into whether these can lead to PD improvement at all and shed light on whether targeting the oxides is worth pursuing. Should this direct targeting of oxygen species prove to be an unviable strategy, targetting the pathways producing them may be a better and logical next step. One such pathway worth targeting would be the inhibition of either MPP or PAR1. A benefit of this approach, over the use of compounds like L-NAME, could be the reduced likelihood of systemic side effects occurring. MPP and PAR-1 inhibition in a model setting has already shown to lead to reductions in both NO and ROS output by microglia there appears to be some promise to this.

A last option when it comes to managing the detrimental effects of α-synuclein microglia interaction could be to steer the subtype population composition within the CNS. This could allow for moving away from the apparent undesirable M1 subtype and towards a seemingly more desirable M2a or c subtype. This would most likely have to be accompanied with either depletion and reintroduction of microglia depending on how rapid and extensively subtype switching can occur within the environment of progressed PD. Studies observing the ways that microglia subtype control is orchestrated would help greatly in this endeavour. Upregulation of the previously discussed Jmjd3 protein can lead to higher proportions of microglia cell populations that skew towards the M2 type. Alternatively, deploying IL-4 and/or IL-13 can achieve this as well under model settings, but this is yet to be confirmed in the disease setting of PD. One last way in which, particularly M2a, subtypes switching could be triggered is through raising the levels of cAMP within microglia, potentially through manipulating adenylyl cyclase or phosphodiesterase activity. Regardless of how microglia subtypes can be manipulated *in vitro,* or *in vivo* models' efforts must be made to translate these into human viable applications, as to not run into roadblocks like the blood-brain-barrier or systemic side effects.

Together these differing inhibitors, antibodies and signalling proteins could, with more targeted research, lead to better and more desirable control over microglia related disease progression and pathology in the setting of PD.

Discussion and Conclusion

Having explored various facets of both Alzheimer's and Parkinson's and their pathology relationship to microglia the central question of this piece can now be answered. Are there points of overlap and potential new therapeutic targets or insights to be found in the fields of Alzheimer's or Parkinson's research that may be translatable to the other with a special focus on microglia?

It appears that microglia play a significant role in the development and progression of both Alzheimer's and Parkinson's. The capabilities of microglia as part of the innate immune system might be expected to help slow down disease progression, microglia seem to have more of a role in exacerbating conditions through various pathways. This can occur through exosome shuttling containing NFTs or α-synuclein or cross-seeding between microglia derived proteins and amyloid-β and α-synuclein any of these can increase the rate and range at which damage is done. These pathways are mostly self-perpetuating reactions that can never resolve on their own. Leading to neuronal cell death in areas they are active in leading to further neural degeneration. Microglia are an option for therapeutic targetting when it comes to disease progression, but not one that deals with the symptoms of Alzheimer's or Parkinson's directly.

Between Alzheimer's and Parkinson's there appear to be some interesting pathways that interact in both diseases with microglia that are open for therapeutic targeting. Primarily the activation of the NLRP3 inflammasome, exosome formation, oxygen species productions and subtype roles appear interesting as each of these have studies from both fields exploring options. In the case of the NLRP3 inflammasome, Alzheimer's studies have explored the use of antibodies targeted at the formed ASC specks preventing interactions with amyloid-β to stop aggregates from forming. In the field of Parkinson's, various methods of inhibiting the inflammasome itself have been attempted, from blocking its activity directly through the pharmaceutical compound MCC950 or through promoting degradation of NLRP3 via Ka. Exosome shuttling has also had different approaches tried and tested. The slowing of the spread of tau being attempted through the depletion of the microglia population, while in the case of α-synuclein exosomes through the formation of the exosome itself within microglia getting inhibited by targeting key proteins needed for exosome functionality via pharmaceutical agent GW4869. The production of oxygen species has been of lesser focus within Alzheimer's research. This does not mean that there cannot be any lessons learned from the work done in Parkinson's. While various oxygen species may not be on the forefront of problems within Alzheimer's, Jmjd3 may still be worth investigating. This pathway is involved in, indirectly, controlling the transcription of IL-1β and may thus pose as an option to reduce IL-1β associated cell death and subsequent amyloid-β release. It seems that Alzheimer's and Parkinson's research while having overlapping pathways with microglia whilst having differing approaches to tackling these, leaving room for both fields to learn from the other and attempt to see if these translate over to anything significant. Clear examples like the ones mentioned in this thesis can potentially be translatable between Alzheimer's and Parkinson's, with more to be found.

One thing that both fields seem to neglect, from my point of view, is a focus on microglia subtypes. Often the M1 and M2 types are relegated to little more than a side note or used in a discussion section to help dealing with inexplicable results. To an extend this is not entirely unreasonable as much remains unknown about the exact inner workings of the various subtypes but there is however growing evidence that these subtypes are important. Especially seeing as distinct behaviours and pathways are attributed to the various subtypes. Another point of potential improvement is with regards to the limited scope of the studies. The effects of an antibody or pharmaceutical agent are mostly explored with regards to one aspect of a disease which often is the entire focus of a study. Many times, not a single line of text is spent on whether there are any effects on the rest of a studied pathology. While a study should focus on a limited set of variables, it should be remembered that these are complex diseases and not loose symptoms that have no interaction. Treating them as such could delay realizations that a potential treatment may work in one aspect but is not effective, or even complicating, other parts of a pathology.

Despite tireless efforts by researchers from around the world, Alzheimer's and Parkinson's remain some of the most common and impactful neurological diseases and will remain so in the years to come. While advances are being made it seems that sometimes it can be helpful to take a step back and learn from other, at first, seemingly unrelated studies and try to translate some of it into one's own topic. Unexpected advances could not be as far away as they sometimes seem.

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