Review paper

Friend or Foe: How glial cells interfere with prion-like spreading

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Abstract
Prion-like spreading has been considered the prominent cause of disease progression in neurodegenerative diseases. Misfolded proteins like Tau, which causes Alzheimer's, and α-syn, which causes Parkinson's, have been found to be the underlying cause of neurodegenerative diseases. These protein aggregates spread through cells into different regions of the brain inducing the symptoms that are associated with neurodegenerative diseases. Current studies show that there are multiple ways that protein seeds or fibrils spread through cells; tunneling nanotubes, exosomes, and trans-synaptic transfer. Here we discuss these forms of transfer and how they work. In addition, we discuss how microglia and astrocytes react to different forms of seed transfer as they attempt to control the diseased environment. Microglia and astrocytes are glial cells that work to regulate the brain's environment and protect it from foreign material that can cause potential harm. In neurodegenerative diseases, these cells tend to be overburdened as they work to eliminate the rapidly spreading protein seeds. Microglia and astrocytes have been observed to play a controversial role in neurodegenerative disease as a growing body of experiments show that they either assist or restrict disease progression. Further research on this topic is necessary to determine the role of microglia and astrocytes in prion-like spreading and to formulate potential methods that can be used to halt prion protein spread so as to slow or even stop the progression of disease.

Keywords
Protein transfer, Microglia, Astrocytes, Protein degradation, α-syn, Tau, Tunneling nanotubes, Exosomes, Trans-synaptic spread, Alzheimer's disease

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1 Introduction

1.1 Neurodegenerative diseases

Parkinson's and Alzheimer's disease are two of the most common neurodegenerative diseases in the world. In the United States, Parkinson's disease is the second most common neurological disorder among older adults (1). Parkinson's is an idiopathic disease that affects the substantia nigra and basal ganglia regions of the brain due to the accumulation of the α-syn protein, thus making it highly progressive (1). A loss in dopaminergic neurons in the substantia nigra causes the symptoms of Parkinson's (1). These symptoms include rigidity, loss of balance, postural instability, hypomimia, and speech impairment, among others (1). People who are at risk of Parkinson's include those with a family history of Parkinson's, exposure to hydrocarbon solvents, working in industrial areas that use copper, manganese or lead, and high intake of iron and/or anemia (1). Parkinson's does not have a cure. Current treatment methods include therapies based on exercise, education, speech and nutrition (1). Pharmacological treatments consist of drugs that increase dopamine levels to control symptoms (1). Like Parkinson's, Alzheimer's disease is also highly common, with 6 million people, mostly older, in the United States living with this condition today (2). Alzheimer's is a form of dementia that affects the hippocampus as the pathogenic form of the neurodegenerative protein Tau accumulates in this region (2). Since the hippocampus is responsible for memory, damage to this area causes symptoms like memory loss, cognitive issues, language impairment, poor judgment, and change in personality, among others (2). The risk factors of this disease include family history, cardiovascular disease, age, metabolic disease, and traumatic brain injury (2). Similar to Parkinson's, there is no cure for Alzheimer's disease. Patients with the disease can take part in therapies that improve cognitive ability or focus on daily activities, such as gardening, that frequently stimulates cognitive function (2). There are pharmacological treatments available to make it easier to cope with the disease. These treatments include drugs that modulate select neurotransmitters in the brain to decrease the severity of symptoms (2). Both diseases begin progressing well before symptoms begin to manifest, making it more difficult to administer treatments before impairment occurs. In addition, the brain's immune response fails to sequester and destroy proteins like alpha-synuclein (α-syn) and Tau, which in turn allows the diseases to progress. Further research on these devastating diseases is needed to identify the exact cause and possible treatments that will slow or stop their progression.

1.2 Prion-like-spreading

Prion proteins are proteins with a distinct three-dimensional structure (3). They frequently fold into beta sheet rich structures. Prion proteins can aggregate together with non-covalent attractive forces between the amino acids constituting the beta sheets. The new folds add to the original one forming large three-dimensional constructs termed fibrils. Fibrils later are thought to crack and continue again to

create larger stacks. This complete cycle is termed seeding and fibrils that are able to continue this process are subsequently named seeds. Diseases caused by prion proteins are highly contagious and mainly affect the central nervous system (3). Over time, prion-protein fibrils travel to different regions of the brain and cause damage which induces the disease symptoms. The proteins associated with Parkinson's disease and Alzheimer's, α-syn and Tau, have been observed to display a prion-like behavior. Higher levels of Tau or α-syn might be one factor that contributes to this phenomenon. Chaperones; proteins that prevent agglomeration of prion proteins are overwhelmed by the rate of prion protein formation. This, along with the failure of other protein structure regulatory factors, causes runaway aggregation of Tau or α-syn. Another reason why (α-syn) protein accumulation might occur, is because of the malfunction of the autophagy-lysosome pathway. This pathway is responsible for recycling or eliminating certain proteins inside cells, specifically neurons. Oxidative stress potentially affects the lysosomal protein p62, an important regulator of this pathway, creating hyper S-nitrosylated p62. This transformation of a significant regulator of the autophagy-lysosome pathway leads to protein aggregates being released into the extracellular environment as the aggregates are unable to undergo autophagy, eventually resulting in their spreading. Regardless of the reason by which protein aggregation occurs, the formation of seeds seems to be the main reason for its progression. Recently, a study by Yemima et. al. demonstrated this phenomenon by delivering nanobodies into neurons to dismantle α-syn fibrils (4). Results showed that the nanobodies kept α-syn pathology in check, thereby attributing protein fibrils as a cause, rather than a consequence of disease.

Additionally, Parkinson's and Alzheimer's present a distinct pattern by which disease spreads through the brain, suggesting prion-like seeding from one cell to another. Multiple ways by which neurodegenerative proteins might spread through the brain, have been described. These include Tunneling-Nanotubes (TNTs), exosomes, and trans-synaptic spread. TNTs provide direct pathways connecting multiple cells over a long distance. Unlike TNTs, exosomes allow for the indirect spread of protein seeds to cells. Exosomes are extracellular vesicles that are taken up by neighboring cells and have been observed to transport protein aggregates. Trans-synaptic transfer occurs when proteins pass through neurons and are released into the environment at the synapse, freely or through exosomes. Prion-like spreading is difficult for the immune system to manage since multiple factors such as, the irreversibility of misfolded protein assemblies, the efficiency by which precursor polypeptides are recruited into aggregates, and the clearance of the aggregates, determine the efficiency of spread (3).

1.3 Glial Cells

Glial cells are the non-neuronal cells of the nervous system. Initially, scientists thought of them as static bystanders when compared with active neurons. This is reflected by the name

they were assigned during the 19th century: Glia from the Greek word for “glue”. It has since become evident that Glia cells’ functions exceed that of simply giving the brain its shape. Their role is to examine the environment for any foreign particles or abnormal proteins using specialized receptors and eliminate them to ensure there is no harm to the brain. There are three types of glial cells: microglia, astrocytes, and oligodendrocytes. Microglia are part of the immune system. They develop in the embryonic sac and specialize in eliminating pathogenic particles and dead neurons. Astrocytes have a similar function to microglia as they regulate the chemical microenvironment (5). Oligodendrocytes are responsible for maintaining the myelin sheath around the neurons. One prominent function of microglia and astrocytes during neurodegenerative diseases is the phagocytosis of surrounding detrimental material (6). When glial cells encounter pathogenic particles, they actively direct them into their soma. Once the material is absorbed, the cells can process them in dedicated organelles called lysosomes (5). Packed with enzymes and operating at low pH levels, these organelles make phagocytosed particles digestible. This process is essential when glial cells participate in neurodegenerative diseases like Parkinson’s and Alzheimer’s. Microglia and astrocytes have been found to clear significant amounts of the misfolded proteins that cause these neurodegenerative diseases. On the other hand, for example in Parkinson’s, glial cells are in close proximity to disease progression. Parkinson’s typically begins at the brain stem and spreads out to different parts of the brain. This can be modeled in mice in various distinct pathways, some of which can be predicted computationally. An accurate prediction of the route by which α-syn spreads through the mice brains could be modeled using the expression of two genes, *Irrk2* and *Gba*, that are predominantly expressed in glial cells (7). Glial cells work hard to stop the progression of neurodegenerative diseases; paradoxically however, they could end up initiating a counteractive overwhelming neuroinflammatory response, which could aid progression (4).

2 Discussion

2.1 Mechanisms of spreading from neuron-to-neuron

As discussed above, one theory on the progression of neurodegenerative diseases is the presence of disease associated protein aggregates in prior healthy neurons. Apart from the idea that these aggregates form inside every neuron individually, new lines of evidence suggest an important role in the transferring of these aggregates from neuron -to -neuron. These transferred aggregates initiate further protein accumulation, referred to as seeds. Here we summarize extra and intra cellular highways which the seeds most prominently hijack, to access healthy neurons. The methods of spreading include tunneling nanotubes, exosomes, and trans-synaptic transfer.

2.1.1 Tunneling Nanotubes

Tunneling Nanotubes (TNTs) are thin tubes that possess actin and connect cells from a long

distance to communicate and transfer various cellular organelles. TNTs have an average diameter of 200 nm and an average length of 20-100 um (8). There are various types of TNTs that specialize in the transfer of certain materials. Thin and open-ended TNTs effectively connect cells together (8). Closed-ended TNTs with a gap junction are useful for transferring electrical signals and small molecules between cells. Close-ended thick TNTs consisting of tubulin and actin allow cell communication from the ends of the tube. Close-ended TNTs that protrude into a recipient cell but have not yet fused to it have been observed as well (3). These forms of TNTs assist with the sharing of several resources that are necessary for the proper function of cells. *In vitro* studies have shown that TNTs facilitate the transporting of appropriate organelles like mitochondria or lysosomes to impaired cells (8). A study by Rostami et al, provided evidence that TNTs support glial cells in the same manner as healthy astrocytes and aided other stressed astrocytes in an environment containing aggregated proteins, by transferring healthy mitochondria through the tubes (9). Although TNTs serve to support cell health, they are also capable of transferring pathogenic material such as misfolded proteins. Aggregated protein fibrils travel through TNTs into the cells that they connect to (10). This method of spreading can be observed with Tau and α-syn, the protein aggregates that accumulate during Alzheimer's and Parkinson's disease. In neuronal cell lines and primary neuronal cultures, Tau fibrils associated with TNTs that formed between either cell (11). Furthermore, in this *in vitro* setting, exposure to exogenous Tau species increased the formation of TNTs. Similar results have been shown in the context of a Parkinson's cell culture model. Primary neurons also transfer α-syn aggregates to neighboring neurons via TNTs (12). This was observed to take part in similar levels of free and lysosomal packed fibrils. More interestingly, α-syn fibrils were shown to escape lysosomal structures inside the recipient cells. Thereafter, these free α-syn species induced further protein accumulation, confirming that they did not lose their seeding abilities during the transfer process (13). Whether this is the case for Tau protein as well has yet to be answered. Taken together, TNTs potentially play an important role in transferring seed competent protein aggregates to the neighboring neurons. One important fact to note is, although well researched in *in vitro* cell culture setting, there is no direct evidence of TNTs *in vivo* in the brain so far.

2.1.2 Exosomes

Exosomes are extracellular vesicles that are 40-100 nm in diameter (14). The primary function of exosomes is to transport materials between cells. Exosomes are composed of tetraspanins, adhesion molecules, enzymes, scaffolds, RNA-binding proteins, RNAs, DNAs, and complex glycans (14). Utilizing these functions, exosomes are able to transfer protein oligomers and RNA (15). The proteins replenish and maintain cells while RNA is used in the process of protein formation. Exosomes assist glial cells equally. *In vitro* studies have

shown that microglia secrete exosomes when under stress during disease or injury. Exosomes have been shown to worsen cellular conditions in a diseased environment because they contribute to the spread of misfolded protein oligomers. Wang et. al., demonstrated this process through examination of microglia that were exposed to α-syn (15). When under stress, microglia initiate a neuroinflammation response which promotes the release of exosomes (15). Microglia that were inefficient in degrading α-syn released exosomes to other cells. These exosomes contained undigested oligomers, thereby promoting the spread of pathogenic proteins (15). A study by Danzer et al., using an *in vitro* model of an exosomal pallet exposed to α-syn, showed that exosomal α-syn was more likely to be taken up by other cells and hence considered to be more toxic (16). Both studies showcase that exosomal transfer of protein aggregates can cause neuronal injury since neurons are capable of taking up exosomes. Wang et. al, presented suggestions to stop this mechanism of spread (17). Their *in vitro* study showed exosomal Tau developing in a cell culture. The results showed that depolarized neurons induce the release of exosomal Tau, highlighting the importance of proper neuron function and activity (17). Current treatments for Alzheimer's disease, caused by Tau protein, include drugs that modulate neurotransmitters to keep neurons active. This type of treatment is likely to counter the spread of protein aggregated through neuronal exosomes since it promotes neuronal activity.

2.1.3 Trans-synaptic

In neurons, the synapse is the significant site of communication. The synapse includes the presynaptic terminal, located at the end of a neuron, and a post synaptic terminal, located at the start of a neighboring neuron. There are two forms of material transport through cells. Anterograde is when substances travel through a neuron with the flow of the electrical current and get transferred from the axon terminal over the synapse to a neighboring neuron's dendrites (18). Retrograde transport occurs vice versa when substances go toward the cell body through the axon from the synapse (18). In addition to signals, materials like proteins which are essential for the recipient cell also travel through the axon terminal. This makes trans-synaptic transfer a target for aggregated proteins to spread to other cells. Hijacking the axonal transport system of neurons, protein fibrils can bridge large distances to reach yet unaffected areas of the brain. Many *in vitro* studies have shown that α-syn can travel through synapses on their own or through lysosomes that carry them across the cell body. It was observed that the transfer of a large quantity of α-syn occurs through retrograde transport (18). More interestingly, the release of protein seeds from neurons was associated with its electrical activity (18). This suggests that it might be tightly coupled to the release of neurotransmitters at the synaptic site. More evidence for the synaptic involvement comes from spatio-temporal observations of affected brains. Protein accumulations seem to follow a predictive pattern during Parkinson's and Alzheimer's disease (18). This pattern

significantly correlates with the neuronal interconnectivity of these brain areas. Furthermore, this form of spreading becomes more prominent in areas with a high concentration of neurons. As activity goes on in the brain and material continues to travel through neurons, aggregated proteins also spread through the synapses. Remarkably, this kind of protein fibril transfer can cover rather large distances. New research suggests that fibrils entering the body by regular ingestion can travel all the way from the gut to the brain, potentially using this transfer route (19).

2.2 Glia cells' involvement in spreading

Glia cells generally are meant to support neurons. However, whether they are able to do so during neurodegenerative diseases remains to be answered. Different lines of evidence point in either direction, which we will discuss in more detail below. Overall evidence that astrocytes and microglia might modulate disease progression comes from prion protein injection models. Interestingly, astroglial changes in mitochondrial and ribosomal transcripts precede actual disease onset as measured by electroencephalograms, the neuronal translatome and immunohistochemistry (20). Particularly, in astrocytes, the gene coding for the chaperone proSAAS was downregulated before disease onset. This chaperone is also found in human AD brains, associated with protein aggregates (20).

In a diseased environment, microglia work in subgroups to carry out various tasks in an attempt to reduce disease. An *in vivo* study by Jessy et al., 2022, made use of single cell RNA sequencing and observed that microglia were more active in a model of prion injection. Transcription alterations, which included less expression of homeostatic markers, occurred in order to trigger more active states across populations of microglia (21). The increased expression of markers such as *Lyz2, Apoe, Tyrobp* and *Irf8* are related to microglia functions such as phagocytosis, proliferation and cytokine signaling. Further sub clustering identified 5 main microglia populations of which one was described as being phagocytic (21). This genetic signature may describe a microglial subtype that is mainly responsible to keep protein aggregate spreading in check. These microglia seem to have a high protein clearance machinery. One of the identified subclusters revealed a remaining population of homeostatic microglia (21). This subgroup is associated with housekeeping functions such as protecting neuronal function and could be important for the survival of neurons during disease. This group of homeostatic microglia present in the study was observed to undergo transcription alterations and decreased in relative frequency (21). This may facilitate neuronal dysfunction during disease progression. Moreover, to examine the extent of microglia significance, an *in vivo* study by Barry et al., where the *Csf1* gene was altered so that the development of microglia would be hindered in mice, analyzed the effects of prion disease in the absence of microglia (22). The study found that mice without microglia did not survive as long as mice with their functional set

of microglia. This shows that microglia are vital during prion infection. Although there was less accumulation of prions found in mice with the altered *Csf1* gene, astrocytes appeared to have become overly reactive and engaged in excessive synapse pruning (22). These results show that microglia help protect neurons from over activated astrocytes in this mouse model of prion injections and that microglia also indirectly protect neurons in addition to their function of directly removing disease associated protein aggregates. Interestingly, not only evidence from prion infection, but also from aging humans points toward a role for glial cells during Tau spreading. An elegant study correlated Tau-PET (a method to detect Tau accumulation in living humans non-invasively) landscapes to the genetic signature of the human brain atlas (23). The results suggested that SLC1A2; the protein product of which clears the excitatory neurotransmitter glutamate from the extracellular space at synapses in the central nervous system and the glial marker APOE were strongly expressed along the route of Tau spread in aged humans.

2.2.1 TNTs

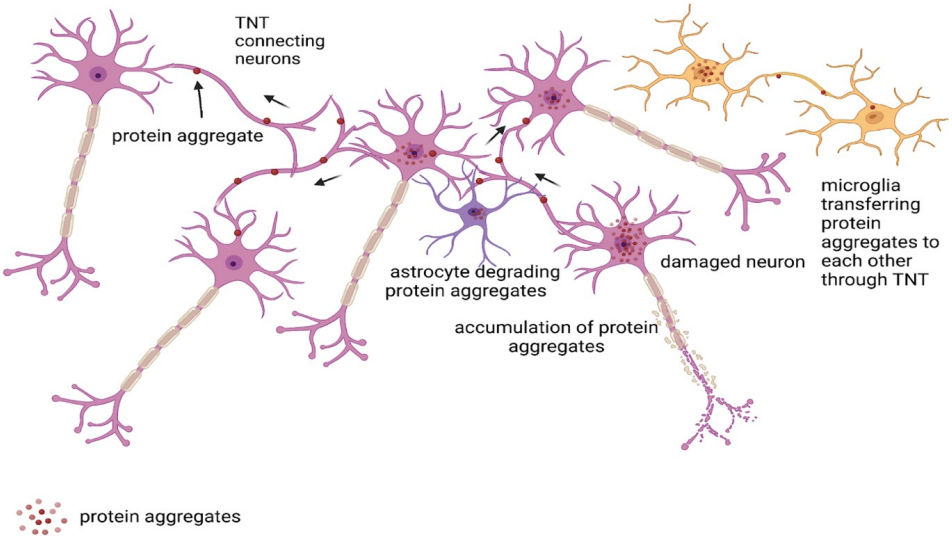

Figure 1: TNTs and their effect on the cellular environment. Created with biorender.com

The study of tunneling nanotubes (TNTs) in the brain is a relatively new field of research. Although TNTs form between neurons *in vitro*, there is little evidence that glia cells interfere with that process. However, glia cells can form TNTs with each other, which could potentially help in managing overwhelming protein amounts.

Microglia

Many studies have shown that microglia form TNTs among themselves and other cells. An *in vitro* study demonstrated that microglia form TNTs when aggregated protein fibrils, like α-syn, were present in the environment (17). It was noted that the function of the TNTs between microglia was to transfer functional material, like mitochondria, to microglia overburdened by α-syn accumulation, which could help prevent cell death (17). This mechanism supports microglia as they attempt to restore homeostasis in a diseased environment. In addition to transferring microtubules that support the cell, microglia transfer α-syn fibrils to other microglia where the aggregates were efficiently degraded (17). The transfer of misfolded proteins allows the work of degradation to be distributed among microglia so that cells don't undergo stress by attempting to degrade large amounts of aggregates. Overall, TNTs among microglia assist microglia while, at the same time, they initiate their immune response as they alleviate stress, which allows them to be more efficient at clearing protein aggregates.

Astrocytes

Similar to microglia, astrocytes play a role in scanning the environment for harmful material like misfolded proteins. Studies have shown that astrocytes asssist with the degradation of full length and cleaved α-syn particles and are not efficient in spreading aggregated proteins (17). This has been demonstrated in quantitative *in vitro* models presenting the formation of TNTs between astrocytes and neurons. α-syn was able to be transferred from one cell to the other. However, this happened significantly more efficiently from neuron to astrocyte. The transferred α-syn was found in the astrocytic lysosomal compartment, stimulating the hypothesis that astrocytes were relieving affected neurons of misfolded protein tendrils with the help of TNTs. Although astrocytes can clear tertiary forms of aggregated proteins, an *in vitro* model, which consisted of human embryonic stem cell derived astrocytes exposed to α-syn oligomers demonstrated that the accumulation of misfolded proteins causes TNTs to form among astrocytes (9). It was observed that α-syn oligomers were not efficiently degraded by astrocytes and were instead trapped in their lysosomal compartment. Furthermore, the accumulation of α-syn in this method induced TNT formation, which assisted in the spreading of aggregated proteins (9). TNTs give aggregated proteins access to widespread areas of cells which, when accumulation occurs, have been observed to cause cell death. The role of astrocytes during TNT spread needs to be further studied. Conclusive evidence is lacking on whether astrocytes are beneficial or harmful during TNT spread in the diseased environment.

2.2.2 Exosomes

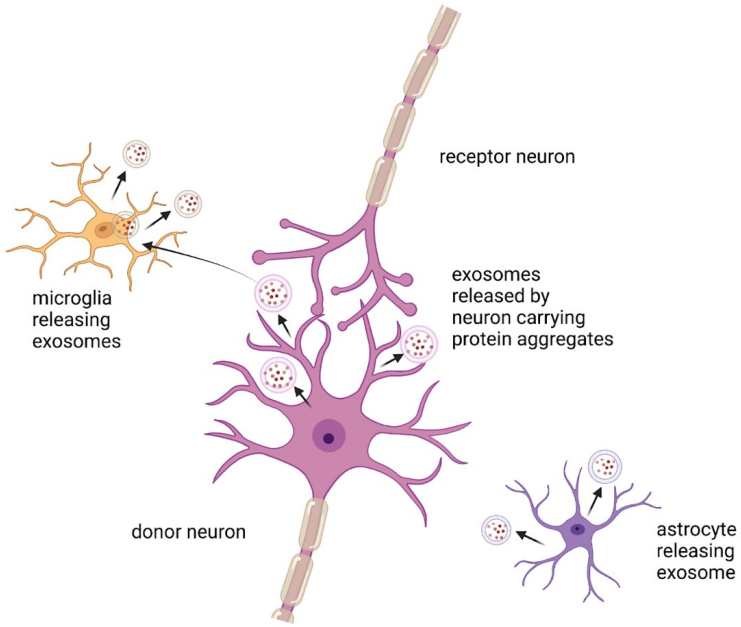

Figure 2: Exosomes containing protein aggregates being released by the neuron, microglia, and astrocyte contributing to the spread. Created with biorender.com

Glia cells can uptake seed containing exosomes. Here we discuss how this may interfere with the transfer of misfolded proteins from neuron to neuron. Also, glia-cells may be a source of seed competent exosomes. Therefore, studying them as a driving force of transfer may prove interesting.

Microglia

An *in vitro* study assessed the yield of exosomes when microglia were exposed to Tau protein in a sample of mouse medial entorhinal cortex. The proportion of microglia in the sample was reduced using an inhibitor, PLX3397, to evaluate how this would affect exosome numbers. The results showed that decreased microglia numbers had reduced the expression of inflammatory cytokines, which help reduce stressful conditions in the cellular environment. In addition, it was also found that ATP which leaked from impaired cells in the diseased environment stimulated microglia and primed them into emitting exosomes, which in turn, facilitated the spreading of Tau. Ultimately, ablating microglia decreased the expression of exosomes and concomittantly reduced the dispersion of aggregated protein (16). Similarly, exosomes distributed by microglia spread α-syn in Parkinson's disease. This study used an *in vivo* model where a

stereotaxic injection of exosomes was placed into the mouse striatum. The authors observed the spread of α-syn after microglia numbers were reduced. They found that the transmission of α-syn was decreased significantly (15). In line with this observation, Wang et. al, demonstrated this process through examination of microglia that had been exposed to α-syn (15). When under stress, microglia initiated a neuroinflammation response which promoted the release of exosomes (15). Microglia that were inefficient in degrading α-syn released exosomes to other cells containing undigested oligomers, thus promoting the spread of pathogenic proteins (15). Similar results were obtained by Xia et al. They worked with exosomes isolated from PD brains and administered them into the mouse striatum. They showed that microglia ablation resulted in decreased movement of α-syn seeds initially contained inside the isolated exosomes. Furthermore, their *in vitro* results suggested that microglia released these protein fibrils after phagocytosis. These released fibrils were able to initiate pathological abnormalities in cultured neurons (24). Overall, the results from both studies that used common aggregated proteins found in the most prominent diseases, Alzheimer's and Parkinson's, showed that microglia exosomes, although being part of an immune response, cause more harm in a diseased environment because they assist with the spread of aggregated proteins.

Astrocytes

Astrocytes also partake in exosome release during neurodegenerative diseases (25). Astrocyte exosomes are beneficial in a diseased environment. According to an *in vitro* model, when the authors identified RNA expression in exosomes from astrocytes and microglia, astrocyte exosomes played a role in metabolic balance (ubiquitin-dependent protein balance) in neurodegenerative diseases (25). Astrocyte exosome regulation of the ubiquitin-dependent protein is significant as this protein assists with the degradation of other proteins, making it facilitatory in an environment of misfolded proteins (25). However, there is also evidence that astrocyte exosomes worsen the progression of neurodegenerative disease. An *in vitro* study demonstrated that when astrocyte exosomes were separated from the plasma of Alzheimer's patients, the complement effector proteins secreted by these astrocyte exosomes were deleterious to neurons in the last stages of neuroinflammation of Alzheimer's. It was postulated that astrocyte exosomes transferred *C3b* to neurons which generated *C5b-C9 TCC,* that, in turn impaired neuronal function (26). Since the role of astrocyte exosomes in neurodegenerative diseases continues to be debated, more studies regarding their function and impact need to be performed to determine whether astrocyte exosomes are conducive or unconducive in neurodegenerative disease.

2.2.3 Trans-synaptic Transmission

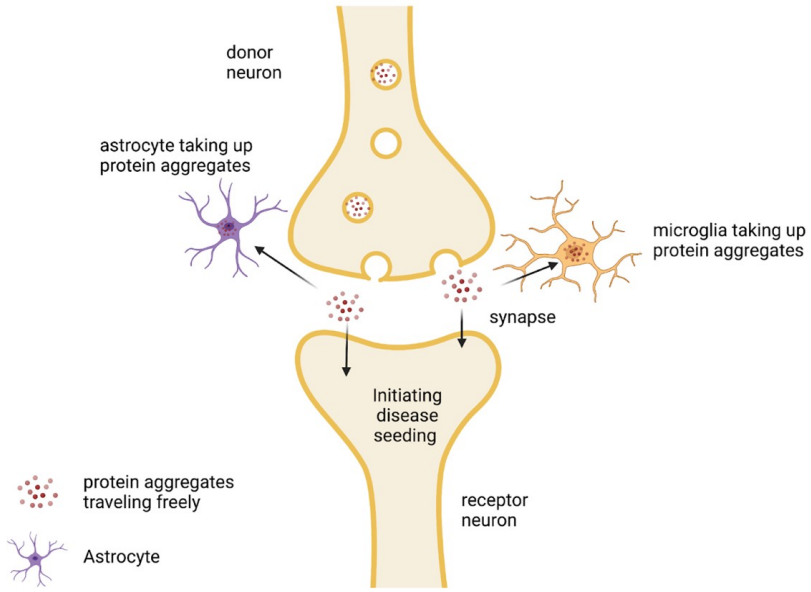

Figure 3: Trans-synaptic transfer of protein aggregates occurring in a neuron synapse as microglia and astrocyte take up the aggregates. Created with biorender.com

Microglia

Trans-synaptic transfer of proteins occurs when proteins travel through a neuron and reach the site of connection between another neuron; called the synapse. This form of transmission results in free forms of proteins being released into the environment and proteins being released through exosomes. Neuronally released α-syn exists mainly in an activity dependent manner suggesting that it originates from the synapse (27). Microglia can access this extracellular α-syn and ingest it by phagocytosis. Although evidence for direct receptor mediated phagocytosis of α-syn is lacking, some receptors have been identified which mediate this uptake. Predominantly, the microglial pattern recognition receptors of the toll-like family, TLR2 and 4, are involved in this process. While TLR4 (24) seems to mainly mediate phagocytosis from neuronal released α-syn that overexpress this protein, TLR2 has been shown to be primarily active in phagocytosis of higher order molecules such as α-syn oligomers and fibrils (28). Interestingly, the processing of α-syn and its degradation depend on TLR4 signaling. Furthermore, Triggering Receptor Expressed on Myeloid Cells 2 (*TREM2*) is implicated in synucleopathies. Although evidence of direct interaction with α-syn is lacking, *TREM2* loss of function mutations (e.g. R47H) were identified as a genetic risk factor for PD. More

importantly, *in vitro* and *in vivo* knock-down/out has been shown to worsen α-syn pathology and drive neuroinflammation (29). On the other hand, the FcyRIIB receptor on microglia seems to inhibit phagocytosis upon binding to fibrillar forms of α-syn. This may boost α-syn spreading towards other neurons when microglial protein removal is impaired by FcyRIIB signaling (30).

An *in vivo* study conducted by Choi et al., demonstrated the release of α-syn by neurons and the response initiated by microglia (24). The study showed that those microglia took up the α-syn released by neurons and began digesting it (24). In addition, the authors pharmacologically depleted the microglia population, which resulted in an increase in the spread of α-syn, in turn leading to further progression of the disease (24). Similarly, George et al., observed the effects of depleted microglia in an α-syn diseased environment among neurons and found a high spread of protein aggregates (20). Another study that had been conducted by Lee et al., used inflammatory processes to reduce microglia function, which led to an increase of protein aggregate spreading (31). An *in vivo* study by Bae et al., utilized antibodies specifically designed for microglia to help them efficiently phagocytose α-syn (32). The results showed that disease progression significantly slowed down the progression of disease and reduced astroglial α-syn burden (32). Overall, microglia facilitated the decrease in this method of spreading.

Astrocytes

Akin to microglia, astrocytes can also mount a similar response to trans-synaptic spread. With an *in vivo* model, Maté de Gérando et. al., found astrocytes with Tau protein in a protein aggregate overexpressed environment (33). In addition, certain populations of astrocyte species-initiated apoptosis after the uptake of large amounts of Tau (33). This evidence demonstrated that astrocytes attempt to clear protein aggregates to stop the spread of disease. A study by Loria et. al, evaluated the efficiency of transfer of α-syn between different cell types (astrocytes, neurons) using an *in vitro* model (25). They found that the transfer of α-syn from neurons to astrocytes was efficient and that the transferred proteins were found in the lysosome compartment of the recipient cell (25). A key observation by Yunseon et al, demonstrated that utilizing astrocytes from the ventral midbrain helped stop the spread and misfolding of α-syn in Parkinson's (34). Astrocytes take time to recognize the accumulation of α-syn in a diseased environment until the disease has greatly progressed whereas, astrocytes from the ventral midbrain are already exposed to the most diseased part of the brain during Parkinson's, hence they are prepared to initiate a response. Hence, it is tempting to speculate that introducing or implanting active astrocytes from the ventral midbrain to other parts of the brain may help reduce the accumulation of misfolded α-syn. These astrocytes use paracrine factors to eliminate pathogenic α-syn (34). This shows that astrocytes play a prominent role uptaking and degrading protein aggregates from affected neuronal cells. This

reduces the stress on neurons in a diseased environment. Multiple studies support the same claim that astrocytes are just as efficient as microglia in degrading protein fibrils, because of their productive degradation rate (31).

3 Conclusion and Outlook

Overall, the mechanisms by which protein aggregates spread include tunneling nanotubes, exosomes, and trans-synaptic transmission. Tunneling nanotubes are created by cells transporting functional material between them; however in a diseased environment, this process leads to the transport of protein seeds between cells. By performing more studies on how misfolded proteins gain access to TNTs and the possible methods that can be applied to block or eradicate affected TNTs, possible treatments to resolve this aspect of disease progression can be developed. Secondly, exosomes - extracellular vesicles - also transport material among cells. Aggregated proteins tend to be passengers in exosomes, thereby amplifying the progression of spreading among cells. More studies on effective ways of temporarily hindering exosome production in a diseased environment, or targeting and eliminating exosomes containing aggregated proteins before they spread to other cells, are needed for limiting the progression of neurodegenerative diseases. Lastly, trans-synaptic spreading occurs when protein aggregates reach the point of communication between cells and are released into the synapse, either by themselves or contained in exosomes. This form of spreading will need to be researched more to determine the exact causes of the travel of protein seeds through neurons, so that methods can be developed to stop this form of transfer. During these forms of prion-like spreading, glial cells attempt to control the spreading in the microenvironment to preserve neuronal function. Microglia and astrocytes degrade protein aggregates and induce spreading mechanisms to distribute phagocytosis among glial cells to lessen the burden on other cells. In some cases, like microglia exosomes, this may result in more harm than benefit, because there occurs an increase in the spread. An understanding of these forms of protein aggregate spreading is necessary in order to reduce or stop neurodegenerative disease progression. The challenge is to stop such spreading without hindering the cooperative phagocytotic distribution of protein aggregates among microglia or astrocytes; two seemingly opposing objectives.

Since multiple findings on glial cells involvement in prion-like spreading appear to contradict each other, more research is needed to conclude whether glial cells restrict or drive disease progression. Evidence of the influence of glia cells on the exosomal path of seed spreading points toward a detrimental role for glia cells. On the other hand, the complete deletion of microglia accelerates disease progression in mouse models of Parkinson's disease. A signifiant challenge that needs to be addressed in order to reconciliate these different outcomes are the limitations of different disease models. There are many established models to mimic Parkinson's

disease in mice. However, some might be more suitable to investigate the seeding properties of α-syn and how those may be influenced. Most models are characterized by the onset of neuroinflammation in the later stages of the disease. In a general sense, inflammation affects microglia as well as astrocytes negatively. Hence, the time of investigation is likely to be very important when studying glial mechanisms that interfere with seed transmission. In addition, effects that have been described in cell cultures may manifest differently in a living organism. For example, TNTs have been well demonstrated as a pathway of spreading *in vitro* but there is little evidence of this mechanism *in vivo*. Furthermore, to investigate the movement of seeds within the brain, temporal analysis is necessary. This makes it particularly difficult to generate human evidence as one can usually study it only *post mortem*.

Currently, there are a few studies that demonstrate promising forms of treatments for these neurodegenerative diseases. One method considered the role of cholesterol. According to an *in vitro* study by Benjamin et al., when the concentration of cholesterol in mice neurons was increased, the entry of tau into the cells was decreased (35). Cholesterol is a vital part of the cell membrane as it assists with ordering the lipid bilayer, controls the layer's fluidity, and regulates its permeability. Thus, the influence of cholesterol on tau spreading may be used to advantage to develop potential therapies. In addition to manipulating cells, attempts to directly target the receptors of protein aggregates have been made. An *in vivo* study by Caixia et al. tested the influence of the LRP1 receptor on different conditions of α-syn. When α-syn was freely able to bind with LRP1, an increase in spread resulted. On the other hand, when LRP1 was deactivated using *LRP1* siRNA, there was reduced presence, exogenous, and endogenous spread of α-syn in cells.

The progression of Parkinson's disease is described by the prion-like behavior of α-syn fibrils. However, it is worth mentioning that some research challenges this theory by showing that α-syn oligomer toxicity happens independently from endogenous α-syn. The authors of such results rather pinpoint mitochondrial damage as a driving factor for neuronal decrease, having identified dopaminergic neurons as being particularly sensitive to such damage (36). Therefore, it might be prudent to direct the research focus on mitochondrial protection strategies as well, in the search for treatment options for PD.

Taken together, the involvement of glial cells in neurodegenerative diseases is an ongoing area of active research. Emerging evidence indicates that non-neuronal cells not only play a role in the onset, but also in the progression of Alzheimer's and Parkinson's diseases. In this review, we summarized the role of glial cells in the neuronal spread of misfolded proteins such as Tau and/or α-syn.

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