

# Microglial and NLRP3 Activation Is Responsible for Alpha-Synuclein Initiating Dopaminergic Neuronal Pathology in Parkinson's Disease

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## Abstract

Parkinson's disease (PD) is a progressive neurodegenerative disorder that involves the death of dopaminergic neurons in the substantia nigra pars compacta. After neuronal death, the subsequent reduction of dopamine levels in the brain induces motor deficits characteristic of this hypokinetic disorder. Although there is currently no known cause of PD, alpha-synuclein ( $\alpha$ -SYN) appears to have a prominent role in both microglial and NOD-like receptor family pyrin domain containing 3 (NLRP3) inflammasome activation. The NLRP3 inflammasome is a complex that, when activated in PD, mediates interleukin 1- $\beta$  (IL-1 $\beta$ ) release. The consequential release of the pro-inflammatory cytokine IL-1 $\beta$  has been demonstrated to be responsible for neuroinflammation and neurodegeneration in PD. The present review highlights the role of  $\alpha$ -SYN aggregates in PD pathogenesis. The PD  $\alpha$ -SYN preformed fibril animal model permits the specific targeting of  $\alpha$ -SYN-mediated microglial and NLRP3 inflammasome activation in newly designed therapies. Studies using this model suggest MCC950, a potent selective inhibitor of NLRP3 that indirectly blocks NLRP3 activation, and its analogs as potential new treatments to prevent neurodegeneration in PD.

**Keywords:** Parkinson's disease, Microglia, Alpha-synuclein, NLRP3, Neurodegeneration, MCC950

Parkinson's disease (PD) is the second most common neurodegenerative disease worldwide (Ambrosi et al., 2014; Gordon et al., 2018; Su et al., 2008), and affects more than 10 million people globally (Hirsch et al., 2016). Additionally,

an estimated 6,600 new cases of PD are diagnosed annually in Canada (UCB Canada Inc., 2018), and another 60,000 in the United States (Hirsch et al., 2016). Further, Huse et al. (2005) determined the economic burden of PD through an

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analysis of 20,016 patients with PD and an equivalent number of controls. They estimated the annual costs of direct health care per patient with PD in the United States to be \$10,349 in 2002 US currency. Considering that there were approximately 645,000 patients in the United States at the time of the study, the total economic cost for PD was \$23.0 billion per year. This cost consists mostly of productivity loss but also accounts for inpatient care, outpatient care, uncompensated care, and prescription drugs. Due to increasingly ageing population in the United States, Huse et al. (2005) estimated the annual cost of PD to be over \$50 billion by 2040. Therefore, research into the causes and potential treatments of PD has the potential to not only increase the quality of life of patients with PD, but to also mitigate substantial economic strains.

Research into the causes and potential treatments of PD is vital to limit the progression of this disease in present and future generations. For decades, researchers have tried to determine the cause of PD but have so far been unsuccessful. Research, however, has unveiled many cellular processes, proteins, and environmental factors that are now identified as being associated with the disease. Unfortunately, many of the underlying mechanisms are still unknown. However, it has been determined that PD is a multifactorial disease which is the result of both genetic and environmental factors (Su et al., 2008). The primary features of PD are mitochondrial dysfunction, the spreading of activated microglia, the accumulation of  $\alpha$ -synuclein ( $\alpha$ -SYN)-rich aggregates in the form of Lewy bodies, neuroinflammation and neurotransmitter modifications in the substantia nigra pars compacta (SNpc), and the substantial death of dopaminergic neurons (DAN) in this area (Gordon et al., 2018; Hsu et al., 2000; Kaur et al., 2018). Ultimately, these characteristics cause several symptoms, which can be grouped into non-motor and motor-related symptoms. The non-motor symptoms of PD include gastrointestinal dysfunction, sleep issues, and neuropsychiatric disturbances (Dzamko et al., 2016; Wolters, 2009). Patients with PD experience a loss of nerve endings that produce the neurotransmitter norepinephrine ("Parkinson's Disease: Hope Through Research", 2014). Several non-motor symptoms in PD, such as fatigue and abnormal blood pressure, might be explained by this loss of norepinephrine ("Parkinson's Disease: Hope Through Research", 2014). These symptoms often precede motor symptoms (also known as parkinsonism) such as rest tremor, bradykinesia, rigidity, and postural instability (Ambrosi et al., 2014; Dzamko et al., 2016). The primary target of therapeutic strategies is the reduction or elimination of parkinsonism (Mayo Clinic Staff, 2018).

The current most popular and effective treatment for PD is a combination of levodopa and carbidopa (Ambrosi et al., 2014; Mayo Clinic Staff, 2018). However, this treatment focuses only on certain non-motor symptoms and its efficacy is reduced over time ("Parkinson's Disease: Hope

Through Research", 2014; Mayo Clinic Staff, 2018). At the present time, there is no cure for the chronic effects that lead to DAN death in PD, but many treatments and therapies are currently being investigated ("Parkinson's Disease: Hope Through Research", 2014). A treatment is needed that both prevents the development and symptoms of PD and makes PD manageable for the long term. Several studies seek to find new approaches to PD therapeutics (Chen et al., 2018; Gordon et al., 2018; Mo et al., 2018).

The present review focuses on  $\alpha$ -SYN-mediated microglial activation, the pro-inflammatory role of the NLRP3 inflammasome in this process, and the subsequent neuroinflammation and dopaminergic neuron death. The activation of the NLRP3 inflammasome by  $\alpha$ -SYN results in the release of the pro-inflammatory cytokine IL-1 $\beta$ . A current method being investigated for treating PD is to prevent neuroinflammation and the prior release of IL-1 $\beta$  by inhibiting NLRP3 activation. This article explores Gordon et al.'s investigations into the potential use of MCC950 as a preventative treatment of DAN loss and thereby a therapeutic for PD, due to its ability to block the secondary trigger signal required for NLRP3 activation.

## Microglial Activation in PD Pathogenesis

Microglia, the resident immune cells in the brain, have a role in the brain's innate immune response through both surveillance of microenvironmental changes and removal of cell debris and foreign molecules (Kim et al., 2006; Su et al., 2008). The three essential functions of microglia are to sense their environment, conduct physiological housekeeping, and protect against foreign agents (Hickman et al., 2018).

Microglia have been detected in three separate states: ramified, primed, and fully activated (Blaylock, 2017). Ramified microglia are important in repairing surrounding structures following microglial activation (Blaylock, 2017). They counteract inflammation by releasing anti-inflammatory cytokines such as Interleukin 10, Transforming Growth Factor Beta 1 and Interleukin 4 (Blaylock, 2017). A number of events lead to microglia entering the primed state, such as head trauma, bacterial infection, or accumulation of toxic chemicals (Blaylock, 2017). Activation of primed microglia causes a larger hyperreaction than does the activation of other microglia. This hyperreaction results in both prolonged and intense neurodegeneration in the brain as a consequence of inflammatory pathways (Blaylock, 2017). Numerous pro-inflammatory stimuli are responsible for the activation of microglia in the central nervous system (CNS) (Lull & Block, 2010). Among these stimuli are lipopolysaccharide (LPS), disease proteins such as mutated or aggregated alpha-

synuclein ( $\alpha$ -SYN), neuron damage, and environmental toxins such as pesticides and air pollution (Block & Calderón-Garcidueñas, 2009; Block & Hong, 2005; Zhang et al., 2005). These various stimuli may explain why PD is considered to be a multifactorial disease (Su et al., 2008). Upon activation by pro-inflammatory stimuli, microglia undergo morphological changes from resting ramified microglia into activated amoeboid microglia (Kreutzberg, 1996). This activated phenotype is associated with a shift in cellular function and the release of cytotoxic factors to destroy invading pathogens (Lull & Block, 2010). These factors include interleukin- $1\beta$  (IL- $1\beta$ ), tumor necrosis factor alpha (TNF- $\alpha$ ), interleukin-18 (IL-18), interferon gamma (IFN- $\gamma$ ), and reactive oxygen species (ROS) such as nitric oxide (NO) (Chao et al., 1992; Kim et al., 2006; Liu et al., 2005; Si et al., 2004; Suk et al., 2001). Studies show an increase in the levels of IL- $1\beta$  in the brains of patients suffering from PD, which is likely due to the release of these cytotoxic factors from activated microglia (Blum-Degen et al., 1995). The release of pro-inflammatory and cytotoxic factors results in inflammation (Coll et al., 2015). DAN demonstrate a unique sensitivity to inflammation and oxidative stress that is not seen in neurons in other brain regions such as the hippocampus or cortex (Blaylock, 2017). Additionally, microglia in the brain may be activated early in the disease process and remain primed following this activation (Tansey & Goldberg, 2010). Therefore, the neuroinflammation and oxidative stress on the surrounding vulnerable neurons may be enhanced through this process. Dopaminergic neurodegeneration is mediated through microglial superoxide release and phagocytosis (Kim et al., 2006). Activated microglia participate in this progressive degeneration of DAN through the phagocytosis of degenerating neurons in the early apoptotic stages (Sugama et al., 2013)

## $\alpha$ -SYN Activates Microglia

$\alpha$ -SYN is a 140-amino acid-protein predominantly located in the presynaptic terminals of neurons under physiological conditions (Blaylock, 2017). Dzamko et al. (2016) examined the anterior cingulate cortex, one of the brain regions pathologically affected in PD, and reported increased  $\alpha$ -SYN during the early stages of PD prior to both pathological Lewy body formation and neuronal loss. This identifies  $\alpha$ -SYN as a possible early pathological indicator of PD.

A defect in microglial internalization and degradation of  $\alpha$ -SYN leads to the accumulation of extracellular  $\alpha$ -SYN (Hickman et al., 2018). Extracellular aggregated human  $\alpha$ -SYN activates microglia, as shown by Zhang et al. (2005), where microglia treated with  $\alpha$ -SYN displayed morphological changes characteristic of activated microglia. Microglia mediate host defense against aggregated  $\alpha$ -SYN and become proinflammatory once

recruited to the area of  $\alpha$ -SYN deposits (Hickman et al., 2018). Microglial activation is further enhanced by the phagocytosis of  $\alpha$ -SYN by microglia, leading to neurodegeneration via NADPH oxidase (NADPHO)-mediated superoxide release, proinflammatory cytokines, or direct phagocytosis of neurons (Codolo et al., 2013; Kim et al., 2006; Zhang et al., 2005). Zhang et al. (2005) observed that NADPHO deficient mice were much more resistant to  $\alpha$ -SYN-induced dopaminergic neurotoxicity compared to wild type mice, and also displayed a reduction in the production of ROS by activated microglia. This data was used to display the direct link between  $\alpha$ -SYN and ROS production that induces DAN loss. Zhang et al. (2005) also demonstrated that the phagocytosis of  $\alpha$ -SYN aggregates is critical for the mediation of microglial activation. Their results showed that cytochalasin D, an inhibitor of phagocytosis, also inhibited  $\alpha$ -SYN-mediated production of ROS in microglia. Therefore, the phagocytosis of  $\alpha$ -SYN by activated microglia appears to play a role in the production of ROS. Aggregated or mutated  $\alpha$ -SYN can also cause mitochondrial dysfunction, which increases oxidative stress (Hsu et al., 2000). Mutated  $\alpha$ -SYN contributes to the neuronal demise and physiological symptoms of PD (Tansey & Goldberg, 2010).

Although some researchers have shed light on how  $\alpha$ -SYN activates microglia and leads to neurodegeneration in PD, the exact mechanisms by which this occurs are not completely understood (Codolo et al., 2013; Gordon et al., 2018). There is evidence to support that the CD36 receptor and one of its downstream kinases, extracellular signal-regulated kinase (ERK), may be involved in this mechanism (Su et al., 2009). Toll-like receptor 2 (TLR2) is expressed in microglia and brain regions pathologically affected by PD (Dzamko et al., 2016). Since increases in TLR2 levels have been associated with increases in  $\alpha$ -SYN, TLR2 may also be involved in the  $\alpha$ -SYN-mediated microglial activation mechanism (Dzamko et al., 2016).

## NLRP3 Inflammasome mediates inflammation induced by microglial phagocytosis of $\alpha$ -SYN

NOD-like receptors (NLRs) are pattern-recognition receptors that are primarily expressed by microglia, astrocytes, and macrophages, and are located in the cytoplasm where they sense intracellular signals (Walsh et al., 2014). NLR family pyrin domain containing 3 (NLRP3) is composed of a N-terminal pyrin domain, a nucleotide-binding domain, and a carboxy-terminal leucine-rich repeat which acts as the sensor in the NLRP3 domain (Gordon et al., 2018; Schroder et al., 2010; Walsh et al., 2014). Once NLRs form complexes with enzyme caspase-1 and an

adaptor protein, they are known as inflammasomes (Gordon et al., 2018; Walsh et al., 2014).

NLRP3 activation is a key contributor to the inflammatory and neurodegenerative processes that occur in PD. NLRP3 activation is a two-step process involving a primary priming signal and a secondary trigger signal (Walsh et al., 2014). The primary priming signal acts through the nuclear factor- $\kappa$ B (NF- $\kappa$ B) pathway to increase the transcription of both NLRP3 and Pro-IL-1 $\beta$  (Walsh et al., 2014). LPS and other microbial Toll-like receptor ligands have been shown to act as the initial signal by priming cells (Walsh et al., 2014). Monje et al. (2003) suggest a mechanism of microglial Toll-like receptor 4 (TLR4) activation by LPS to induce the release of pro-inflammatory cytokines and ROS. The secondary trigger signal mediates the assembly of the NLRP3 inflammasome and the associated activation of caspase-1 (Walsh et al., 2014). The secondary signals include many inflammatory factors such as LPC, ATP, nigericin, prion protein fibrils, and  $\alpha$ -SYN (Freeman et al., 2017; Gordon et al., 2018; Hafner-Bratkovic et al., 2012). Results from Freeman et al. (2017) convey the importance of the NLRP3 inflammasome in mediating microglial LPC-induced IL-1 $\beta$  secretion following LPS priming. On the other hand,  $\alpha$ -SYN-induced release of IL-1 $\beta$  and activation of the NLRP3 inflammasome do not appear to require microglial priming (Gordon et al., 2018). However, priming the microglia with LPS prior to the  $\alpha$ -SYN stimulus produces a much greater response in terms of IL-1 $\beta$  secretion (Gordon et al., 2018). This may be due to the increased expression of pro-IL-1 $\beta$  and NLRP3 through the NF- $\kappa$ B pathway by the priming stimulus prior to the activation by  $\alpha$ -SYN (Walsh et al., 2014).

Once activated, the NLRP3 forms an inflammasome complex with the caspase-1 protease, the adaptor apoptosis-associated speck-like protein containing a CARD (ASC), and the NLRP3 sensor (Codolo et al., 2013; Gordon et al. 2018) (Figure 1). The adaptor protein, ASC, facilitates the interaction of caspase-1 and NLRP3 in the formation of the inflammasome (Schroder et al., 2010; Walsh et al., 2014). Pro-caspase-1 takes on its active form of caspase-1 once it is introduced to the assembling inflammasome (Codolo et al., 2013). The maturation and release of the pro-inflammatory factors IL-1 $\beta$  and IL-18 are mediated through caspase-1 protease activity (Walsh et al., 2014). Although IL-18 is produced in primary mouse microglia as a result of NLRP3 activation by ATP, no IL-18 secretion is observed through  $\alpha$ -SYN-mediated NLRP3 activation (Gordon et al., 2018). On the other hand, NLRP3 activation by both ATP and  $\alpha$ -SYN elicits the production of IL-1 $\beta$  (Gordon et al., 2018; Schroder et al., 2010). The active form of caspase-1 mediates the cleavage of pro-IL-1 $\beta$  into IL-1 $\beta$ , which is then released (Walsh et al. 2014). In addition to the release of these pro-inflammatory factors, caspase-1 triggers pyroptosis – a highly inflammatory form of programmed cell death (Jorgensen et al., 2017). However,

the downstream effect of pyroptosis depends on the upstream stimulus of NLRP3 activation (Gordon et al., 2018). Increased levels of caspase-1, ASC, and IL-1 $\beta$  as a result of  $\alpha$ -SYN-mediated NLRP3 activation are not associated with pyroptotic mechanisms as they are also involved in conventional NLRP3 activation by nigericin (Gordon et al., 2018).

$\alpha$ -SYN has been shown to have a significant effect on the expression of NLRP3 - more so than NLRP1 (Codolo et al., 2013). The expression of NLRP3 compared to NLRP1 identifies NLRP3 as the main inflammasome involved in prompting the downstream pro-inflammatory effects of  $\alpha$ -SYN. Codolo et al. paralleled the action of microglia through the use of what they determined to be a more reliable model that better represents the *in vivo* situation in PD – monocytes. In a comparison between untreated monocytes and those treated with  $\alpha$ -SYN, the latter become enlarged due to the phagocytosis of  $\alpha$ -SYN fibrils ( $\alpha$ Syn F). Phagocytosis of  $\alpha$ -SYN has proved essential for  $\alpha$ Syn F-induced IL-1 $\beta$  release (Codolo et al., 2013). Equivalent amounts of IL-1 $\beta$  are released one hour after stimulation with the NLRP3 activator ATP, and 24 hours after stimulation with  $\alpha$ -SYN (Gordon et al., 2018). The delayed potent inflammatory response due to  $\alpha$ -SYN in microglia is likely due to the phagocytosis of  $\alpha$ -SYN fibrils (Gordon et al., 2018).

Inflammatory processes are broadly influenced by IL-1 $\beta$ , which is one of the most abundant pro-inflammatory cytokines (Dinarello, 2010). The maturation and activation of IL-1 $\beta$  are mediated by the active caspase-1 portion of the NLRP3 inflammasome (Codolo et al., 2013). Increased levels of an activated caspase-1 zymogen can be found in cases of neuroinflammation, such as PD, due to the role of active caspase-1 in activating IL-1 $\beta$ . Similarly, a higher amount of  $\alpha$ -SYN can correlate with increased ASC levels in PD patients. Internalization of  $\alpha$ -SYN mediates NLRP3 activation and ASC release in microglia (Gordon et al., 2018). These mechanisms are supported by the findings that levels of cleaved caspase-1, as well as the adaptor protein ASC, are significantly increased in the brains of patients with PD compared to age-matched controls (Gordon et al., 2018).

## Current and Future PD Therapies

Neurodegeneration of DAN in PD directly decreases dopamine levels ("Parkinson's Disease: Hope Through Research", 2014). Therefore, many of the current medications either increase or substitute for dopamine (Mayo Clinic Staff, 2018). Drugs that increase brain dopamine levels are the most common; however, they make up only one of the three categories of PD drugs. The second category consists of drugs that affect other neurotransmitters in order to reduce PD symptoms related

to motor deficits such as tremors ("Parkinson's Disease: Hope Through Research," 2014). The third category of drugs includes those prescribed to help control the non-motor symptoms of the disease, such as antidepressants, to help with PD-related depression ("Parkinson's Disease: Hope Through Research", 2014). The medications that may be used by PD patients include levodopa/carbidopa, dopamine agonists, monoamine oxidase B (MAO-B) inhibitors, catechol-O-methyltransferase (COMT) inhibitors, amantadine, and anticholinergics ("Parkinson's Disease: Hope Through Research", 2014; Mayo Clinic Staff, 2018). Certain drugs may be used in combination, such as amantadine with levodopa or anticholinergic drugs ("Parkinson's Disease: Hope Through Research", 2014).

The most effective current therapy, levodopa (L-Dopa), only provides symptomatic relief of motor parkinsonism and does not prevent neurodegeneration (Ambrosi et al., 2014). Dopamine is too polar to pass the blood-brain barrier, which prevents it from directly entering the brain (Gregory & Burnham, 2008). On the other hand, L-Dopa is able to pass the blood-brain barrier, where it is then converted into dopamine through the removal of a carboxylic acid group by the enzyme dopa decarboxylase (Gregory & Burnham, 2008; Mayo Clinic Staff, 2018). Carbidopa (also known as lodosyn) prevents the early conversion of L-Dopa to dopamine outside the brain and thus is usually combined with L-Dopa therapy to reduce side effects such as nausea (Mayo Clinic Staff, 2018). This medication may help patients manage problems with walking, movement, and tremor (Mayo Clinic Staff, 2018). However, these symptomatic improvements that patients experience are often short term and are only sustained through gradual dose increases of L-Dopa ("Parkinson's Disease: Hope Through Research", 2014). Long-term treatment with L-Dopa eventually leads to L-Dopa induced dyskinesias, which further reduces motor control and requires its own treatments (Tran et al., 2018). The average time to onset for movement complications after starting L-Dopa treatment is approximately 6.5 years (Shrag & Quinn, 2000). L-Dopa induced dyskinesias can be reduced by lowering the dose of L-Dopa and by taking additional medications such as amantadine (Tran et al., 2018). Although L-Dopa is currently the most effective drug to treat PD symptoms, it becomes less efficacious and results in undesirable symptoms over time. Levodopa is not the ideal treatment option as it does not target the root of the disease. Research has been dedicated to developing new disease-modifying therapies for patients with PD (Gordon et al., 2018; Mo et al., 2018).

MCC950 (also called CP-456,773 and CRID3) is a potent selective inhibitor of NLRP3 that blocks NLRP3 activation (Coll et al., 2015; Mangan et al., 2018). Although it has been determined that MCC950 specifically inhibits NLRP3, the exact molecular target involved in this mechanism is still unknown (Mangan et al., 2018). Gordon

et al. (2018) were able to effectively block ATP and nigericin-mediated activation of NLRP3 in primary microglia using 7.7nM of MCC950, which was shown to be its median inhibitory concentration ( $1C_{50}$ ). The small concentrations at which the downstream effects of NLRP3 activation by  $\alpha$ -SYN aggregates are able to be blocked prove beneficial for the use of this drug or its derivatives as potential therapeutics for PD (Gordon et al., 2018).

Pre-treatment with MCC950 after the priming signal is effective in blocking the secondary trigger signal involved in the formation of the NLRP3 inflammasome (Coll et al., 2015). MCC950 blocks the ability of all known stimuli to activate the NLRP3 inflammasome – one of which is fibrillar  $\alpha$ -SYN (Codolo et al., 2013; Mangan et al., 2018). By blocking the NLRP3 inflammasome activation, MCC950 also prevents the activation of caspase-1 and subsequently, the conversion of pro-IL-1 $\beta$  to IL-1 $\beta$  (Coll et al., 2015) (see Figure 1). This drug is specific to the inhibition of IL-1 $\beta$  secretion, as no inhibition of the pro-inflammatory cytokine TNF- $\alpha$  occurred after treatment (Coll et al., 2015). The selective inhibition of MCC950 is important since it prevents the unwanted side effects of blocking TNF- $\alpha$  directly (Steeland et al., 2018), as well as the manipulation of additional TNF- $\alpha$  pathways (Olmos et al., 2014). The specificity of MCC950 is important in targeting the specific  $\alpha$ -SYN-mediated pathological mechanisms involved in PD. The activity of this drug has been explored in mice models of PD induced by  $\alpha$ -SYN aggregates to test its efficacy in treating the disease (Gordon et al., 2018).

Traditional models of PD are toxin-induced (Polinski et al., 2018). The injection of such toxins into the brain or periphery induce rapid degeneration of DAN in the SNpc (Polinski et al., 2018). Two widely used toxin-treated models are 6-hydroxydopamine (6-OHDA)-induced and 1-methyl-4-phenyl-1, 2, 3, 6-tetrahydropyridine-(MPTP)-induced mouse models (Polinski et al., 2018). These models exhibit well-characterized motor deficits and nigrostriatal degeneration, but in most cases, they fail to exhibit  $\alpha$ -SYN aggregates and Lewy body pathology (Beal, 2010; Polinski et al., 2018). A more recent model of PD that accounts for this is the  $\alpha$ -SYN preformed fibril (PFF) mouse model (Polinski et al., 2018).

PFF mice models are used to mimic the pathological effects of PD, such as the accumulation of Lewy bodies, DAN loss in the SNpc and reductions in striatal dopamine, and the symptomatic motor deficits associated with the disease (Luk et al., 2012). Gordon et al. (2018) performed a variety of motor function tasks on PFF mice models treated with MCC950, in order to investigate the effect of MCC950 treatment on the DAN degeneration involved in PD by  $\alpha$ -SYN aggregates. PFF Mice treated with MCC950 performed better on the rotarod, balance beam, and wire-hang test than untreated PFF mice. Additionally, the MCC950-treated PFF mice demonstrated notably increased dopamine concentrations in the striatum and

significantly more DANs, as indicated by tyrosine hydroxylase (TH), than untreated PFF mice. Since the PFF model specifically targets  $\alpha$ -SYN-mediated DAN loss, Gordon et al. (2018) were able to conclude that PFF mice are protected against this  $\alpha$ -SYN-mediated degeneration of DAN when treated with MCC950 – indicative of a neuroprotective mechanism.

The experiments performed by Gordon et al. (2018) only confirm that MCC950 is effective in the early stages of PD, since they administer the drug before inducing neurodegeneration in the mouse model. Symptoms appear in PD patients only after 60 to 80 percent of DAN in the substantia nigra have been lost ("Parkinson's Disease: Hope Through Research", 2014), which is the point at which these patients would begin seeking treatment. Although this is considered early-onset PD, substantial neuronal death is still present. Therefore, a more accurate model may be a PFF mouse model with approximately eighty percent DAN loss prior to MCC950 treatment administration in order to reflect the human treatment conditions. Since the human system is much more complicated than mice models, human trials must also eventually be done. According to Dr. Woodruff in *Science Daily*, Gordon et al. hope to carry out human clinical trials with MCC950 analogs in 2020 (University of Queensland, 2018). Although additional testing of MCC950 and its analogs are still needed, the potential of this drug is promising. Since it targets the activation of NLRP3 inflammasome, MCC950 is able to prevent the downstream neuroinflammatory and neurodegenerative effects of multiple stimuli characteristic to PD such as  $\alpha$ -SYN, mitochondrial dysfunction, and the formation of ROS. While the present review highlights the implications of  $\alpha$ -SYN in the activation of microglia and the NLRP3 inflammasome, it is important to note that there is a significant amount of other proteins and mechanisms involved in PD pathogenesis, including NLRP3 activators that are blocked by MCC950 action. Gordon et al. (2018) have created multiple analogs of MCC950, since lapsed patents have prevented the commercialization of MCC950 itself. Therefore, additional experiments may be done using these analogs. A benefit of using MCC950 analogs is the potential for their manipulation to create improved chemical characteristics (Gordon et al., 2018).

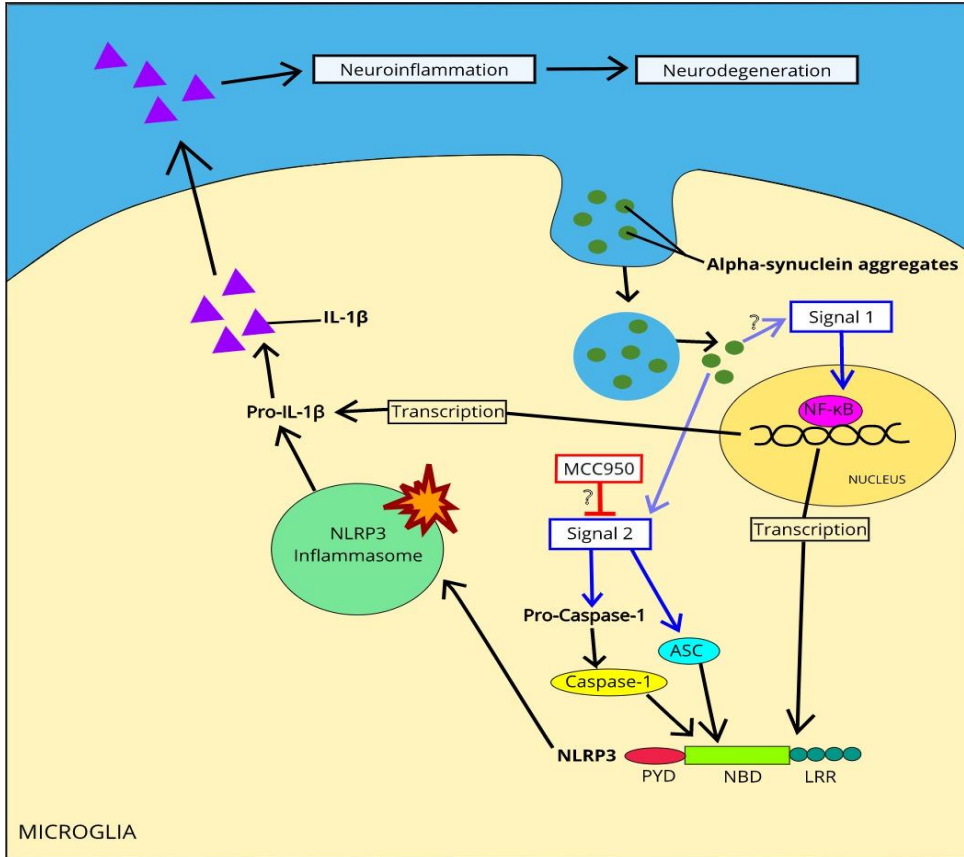
## Conclusion

PD is a widespread neurodegenerative disorder that affects thousands of people in North America every year (Hirsch et al., 2016; UCB Canada Inc., 2018). Due to its large impact on both patient well-being and the economy, many studies are looking into new potential treatment options for this disease (Chen et al., 2018; Gordon et al., 2018; Huse et al., 2005; Mo et al., 2018). However, the mechanisms involved

in the progression of PD must be characterized before more effective treatments can be produced. These mechanisms include the substantial activation of microglia, aggregation or mutation of  $\alpha$ -SYN, neuroinflammation due to pro-inflammatory cytokines such as IL-1 $\beta$ , and neurodegeneration in the SNpc (Gordon et al., 2018; Hsu et al., 2000; Kaur et al., 2018). Although we know these components are involved in PD pathogenesis, knowledge of many of the precise underlying mechanisms are lacking (Codolo et al., 2013; Mangan et al., 2018). As such, future studies should dive deeper into the roots of these mechanisms. One of the mechanisms that needs to be further understood is the activation of the NF- $\kappa$ B pathway by  $\alpha$ -SYN aggregates. Further studies could monitor primary priming signals, such as LPS and other microbial Toll-like receptor ligands, upon stimulation of a cell by different amounts of  $\alpha$ -SYN aggregates to determine a correlation between these factors.

$\alpha$ -SYN mediates both the activation of microglia and the NLRP3 inflammasome, which are key modulators of neuroinflammatory and neurodegenerative pathways. The downstream release of IL-1 $\beta$  is mediated by the phagocytosis of  $\alpha$ -SYN fibrils by activated microglia. Activated microglia have also been shown to phagocytose neurons in the central nervous system – accounting for a portion of the death of DAN. DAN loss results in significantly lowered dopamine levels in the brain and subsequent involuntary motor symptoms that are targeted in therapies.

Current therapeutic options are both limited and unideal for the long-term health of patients with PD. Levodopa (L-Dopa) is presently the most popular and effective treatment for PD symptoms, but it fails to prevent neurodegeneration in the SNpc (Ambrosi et al., 2014). Chronic use of L-Dopa also leads to L-Dopa-induced dyskinesias, which add to the motor deficits already present in the disease and require subsequent treatment (Tran et al., 2018). A current treatment undergoing experimentation is the use of MCC950 or its analogs to block NLRP3 activation and its downstream effects of neurodegeneration (Gordon et al., 2018). Further testing is required in both animal models and humans before this treatment option can be approved. Our lack of understanding of the underlying mechanisms of PD contributes to the lack of current therapies for PD. For example, the contribution of the inflammasome activation to neuronal death pathways has yet to be elaborated (Lee et al., 2017). In addition, a multitude of stimuli are capable of activating the NLRP3 inflammasome, which may indicate the possible involvement of cofactors in the direct activation of the inflammasome (Latz et al., 2013).



**Figure 1:** Illustration of NLRP3 activation in microglia and its involvement in neurodegeneration. Signal 1 and signal 2 represent the primary priming signal and the secondary trigger signal of NLRP3 activation, respectively. Phagocytosis of alpha-synuclein aggregates may act as a priming signal and/or a trigger signal. The exact mechanisms involved in the activation of the NF-κB pathway by alpha-synuclein aggregates is unknown, as indicated by the question mark. Signal 2 mediates the formation of an active NLRP3 inflammasome through the addition of caspase-1 and ASC to NLRP3. MCC950 appears to block the secondary trigger signal involved in NLRP3 activation, but the exact molecular target is still unknown. NLRP3, NLR family pyrin domain containing 3; NF-κB, nuclear factor-κB; ASC, apoptosis-associated speck-like protein containing a CARD; NBD, nucleotide-binding domain; LRR, leucine-rich repeat; PYD, pyrin domain; IL-1β, interleukin 1-β.

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