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CONTEMPORARY REVIEW

Inflammasomes: An Emerging Mechanism Translating Environmental Toxicant Exposure Into Neuroinflammation in Parkinson's Disease

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ABSTRACT

Evidence indicates that complex gene-environment interactions underlie the incidence and progression of Parkinson's disease (PD). Neuroinflammation is a well-characterized feature of PD widely believed to exacerbate the neurodegenerative process. Environmental toxicants associated with PD, such as pesticides and heavy metals, can cause cellular damage and stress potentially triggering an inflammatory response. Toxicant exposure can cause stress and damage to cells by impairing mitochondrial function, deregulating lysosomal function, and enhancing the spread of misfolded proteins. These stress-associated mechanisms produce sterile triggers such as reactive oxygen species (ROS) along with a variety of proteinaceous insults that are well documented in PD. These associations provide a compelling rationale for analysis of sterile inflammatory mechanisms that may link environmental exposure to neuroinflammation and PD progression. Intracellular inflammasomes are cytosolic assemblies of proteins that contain pattern recognition receptors, and a growing body of evidence implicates the association between inflammasome activation and neurodegenerative disease. Characterization of how inflammasomes may function in PD is a high priority because the majority of PD cases are sporadic, supporting the widely held belief that environmental exposure is a major factor in disease initiation and progression. Inflammasomes may represent a common mechanism that helps to explain the strong association between exposure and PD by mechanistically linking environmental toxicant-driven cellular stress with neuroinflammation and ultimately cell death.

Key words: Parkinson's disease; inflammasome; environment; toxicant; neuroinflammation; neurodegeneration.

The clinical progression of Parkinson's disease (PD) is well-documented but we still do not understand how PD is initiated and what factors modify the disease course. The vast majority of PD occurs sporadically with no evidence of a causal genetic lesion (Rajput *et al.*, 1984) and epidemiologic and experimental evidence strongly support a role for environmental exposure in the incidence and progression of idiopathic PD (for review [Cannon and Greenamyre, 2013; Fleming, 2017; Hatcher *et al.*, 2008; Tanner *et al.*, 2011]). Despite these findings, the molecular basis of increased risk resulting from environmental exposure remains poorly characterized. Toxicants released into the environment as the result of human activity accumulate in the air, water, and soil and consist of a variety of synthetic chemical pollutants such as detergents, solvents, and pesticides as well as other industrial by-products such as carbon-dioxide, various particulates, and heavy metals (Zhuang *et al.*, 2015). These pollutants can persist and

pose a challenge to host organisms because the immune system has not evolved mechanisms for managing exposure to newly emerging man-made pollutants. This is highly relevant to progressive age-related neurologic disorders because many toxicants are central nervous system (CNS) permeant, readily crossing the blood-brain barrier (BBB), potentially impacting the health and function of CNS cells over an extended period of time. Based on the likelihood that a high percentage of PD cases are associated with environmental exposure (Costello *et al.*, 2009; Klein and Westenberger, 2012; Liou *et al.*, 1997; Tanner *et al.*, 2011; Wang *et al.*, 2014), characterization of cellular mechanisms engaged by environmental toxicants is expected to reveal key aspects of initiation and progression, improving our ability to diagnose, monitor, and treat the disease.

The mechanisms by which environmental toxicants activate specific inflammatory processes range from the induction of acute necrosis to more discrete cellular pathophysiologies including oxidative stress, protein misfolding, and programmed cell death (Cannon and Greenamyre, 2013). Inflammasomes can mediate the cytosolic response to sterile inflammatory triggers and the induction of inflammasome activity by PD-associated toxicant-driven cellular stress is a rational means by which the CNS might translate environmental toxicant exposure into neuroinflammation. Supporting this premise are recent reports of toxicant-based animal models of PD indicating that genetic inactivation of the inflammasomes suppresses neuroinflammation and is neuroprotective (Lee *et al.*, 2018; Martinez *et al.*, 2017; Yan *et al.*, 2015). Here, we discuss mechanisms of inflammasome activity that are relevant to known cellular pathologies observed in PD. We highlight recent findings related to inflammasomes in the response to PD-associated toxicants, providing a platform for addressing longstanding questions related to the impact of environmental pollution on the initiation and progression of PD.

MECHANISMS AND ORIGINS OF INFLAMMASOME ACTIVITY

Inflammasomes are multi-protein cytosolic complexes that contain pattern recognition receptors (PRRs) that sense both viral and microbial pathogen-associated molecular patterns (PAMPs) and damage-associated molecular patterns (DAMPs). Inflammasomes are organized into three sub-families based on their protein composition: nucleotide-binding domain-like receptors (NLRs), absent in melanoma 2-like receptors (ALRs), and the more recently discovered pyrin family. These core proteins drive caspase-1 catalysis and thereby initiate cytokine maturation, and potentially pyroptosis, an inflammatory caspase-1-dependent subcategory of programmed cell death (Chen *et al.*, 1996). Among the inflammasome subfamilies, the NLRs have been reported in association with sterile inflammation and disorders of the CNS. NLR proteins are defined by three conserved domains: a central nucleotide-binding and oligomerization (NACHT) domain, a C-terminal leucine-rich repeats (LRRs), and either a pyrin domain (PYD) or a caspase activation and recruitment domain (CARD) at the N-terminus and are encoded by 25 currently annotated genes (Ting *et al.*, 2008). Among the NLRs, *NLRP3* is the most widely studied in the context of sterile inflammation and neurologic disease and therefore will be the principal complex discussed throughout this review (Figure 1). For further details, excellent reviews are available describing the cellular and molecular biology of the inflammasomes (He *et al.*, 2016; Hoffman and Brydges, 2011; Schroder and Tschopp, 2010; Sharma and Kanneganti, 2016).

Environmental toxicant exposure causes intracellular damage and stress leading to the elaboration of DAMPs. Here, we will consider the impact of PD-associated environmental toxicants on inflammasome activity within the framework of three well-supported models of inflammasome activation: the channel model (Kahlenberg and Dubyak, 2004), the lysosome rupture model (Hornung *et al.*, 2008), and the mitochondrial-derived ROS (mito-ROS) model (Shimada *et al.*, 2012). The channel model is based on the observation that sterile activating agents gain access to the intracellular complex via translocation into the cytoplasm through the formation of non-selective pores in the cytoplasmic membrane. In these studies, the ATP-gated P2XZ ion channel is modified by Pannexin 1 resulting in the formation of large non-selective pores that are required for caspase-1 activation and IL-1B maturation in the context of ATP-exposed LPS-primed macrophages (Pelegrin and Surprenant, 2006). In more recent studies, this model has been refined through the comparison of multiple inflammasome stimuli. Munoz-Planillo *et al.* identify K^+ efflux as the common requirement of inflammasome activation by multiple agonists and determine that whereas non-selective pore formation is a component of the response to certain stimuli, these pores are not required for others (Munoz-Planillo *et al.*, 2013). The lysosomal rupture model of inflammasome activation accounts for larger particulate toxicant activators of the inflammasomes and is also highly relevant to proteinopathies like PD where toxicants themselves and misfolded proteins can contribute to lysosomal damage (Halle *et al.*, 2008). Lysosome rupture releases a host of proteases into the cytoplasm including cathepsin B. Cathepsin B is able to activate the NLRP3 inflammasome and cathepsin B inhibitors, such as Ca-074-me, can impede NLRP3 activation in human cells exposed to silica (Hornung *et al.*, 2008). Likewise, evidence indicates that PD-associated heavy metals like manganese can cause lysosomal stress and death in glia (Gorojod *et al.*, 2017). Elevated ROS is a major indicator of neuronal distress in PD and a common feature of channelopathies, lysosome distress, and toxicant exposure. ROS has been found to drive an interaction between the LRRs of NLRP3 and the thioredoxin (TRX)-interacting protein (TXNIP), to regulate autophagy and impact PD-associated synucleinopathy through the inhibition of ATP13A2 (Su *et al.*, 2017). In the presence of ROS, TXNIP can become unbound and associate with NLRP3 (Zhou *et al.*, 2010) resulting in the activation of the NLRP3 inflammasome. In TXNIP-deficient animals, both caspase-1 activation and IL-1B secretion are decreased in macrophages following exposure to multiple toxicants associated with NLRP3 activation (Zhou *et al.*, 2011). Indicating that similar processes unfold in the CNS, recent studies in the mouse microglial BV2 cell line model of thrombin-induced inflammation suggest that dampening of the TXNIP/ROS pathway with the ROS scavenger N-acetylcysteine both reduces NLRP3 activation and ameliorates apoptosis (Ye *et al.*, 2017). Independent of the mode of activation, NLRP3 inflammasome formation results in the cleavage of procaspase-1 into its active form. Active caspase-1 can cleave pro-inflammatory cytokines, including IL-1B and IL-18, and/or trigger other caspase-1-dependent processes, such as pyroptosis.

CELLULAR ORIGINS OF INFLAMMASOME ACTIVITY IN THE CNS

Although inflammasomes are best characterized in the peripheral innate immune system, increasing evidence indicates that CNS cells also harbor inflammasome activity. Microglia provide

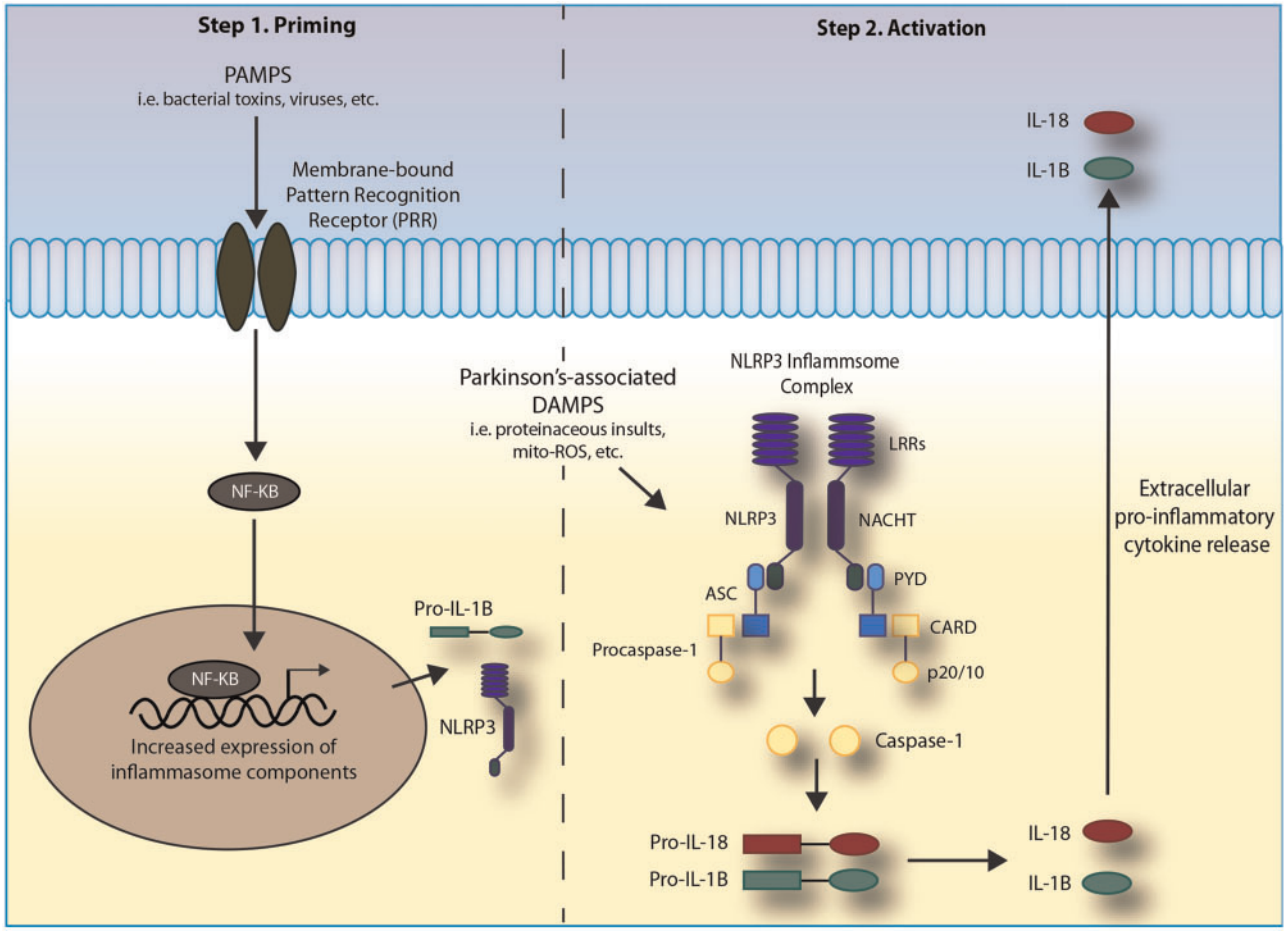

Figure 1. Mechanism of NLRP3 inflammasome assembly. Two major steps are required for the formation of the NLRP3 inflammasome complex, (1) priming and (2) activation. Priming begins when a pro-inflammatory stimulus, such as a pathogen-associated molecular pattern (PAMP) or damage-associated molecular pattern (DAMP), interacts with a pattern recognition receptor (PRR) resulting in alterations in transcriptional programs, including NF-kB activation. Upon activation, NF-kB translocates into the nucleus where it increases expression of key inflammasome-related proteins including pro-IL-1B and NLRP3. A second stimulus is required for formation of the NLRP3 inflammasome complex. Stimuli have recently been identified to include the Parkinson's-associated sterile trigger mitochondrial ROS, among others (see Figure 3). Structurally, the NLRP3 protein is comprised of three main domains—an amino-terminal pyrin (PYD) effector domain, a central nucleotide binding domain (NACHT), and a carboxy-terminal leucine-rich repeat (LRR) motif. Upon activation, the monomeric intracellular NLRP3 proteins interact through the homophilic NACHT domain. Once formed, this complex can interact with the PYD of the adaptor protein apoptosis-associated speck-like protein containing a C-terminal caspase recruitment domain (ASC). The N-terminal CARD domain of ASC then recruits pro-caspase-1 through its CARD domain to form the complete inflammasome complex. Pro-caspase-1 undergoes auto-activation when it binds to oligomerized NLRP3, resulting in cleavage into the p10 and p20 fragments of caspase-1. These active fragments cleave and subsequently activate the pro-inflammatory cytokines pro-IL-1B and pro-IL-18. Once activated, these cytokines are transported out of the cell where they can act in a pro-inflammatory manner.

substantive innate immune monitoring and inflammatory mediation in the CNS and are capable of secreting cytokines to influence local CNS cells and the peripheral immune system (Wolf *et al.*, 2017). Originally characterized in peripheral monocytes, the canonical inflammasome pathway that results in the maturation and secretion of IL-1B is now well-described in microglia indicating that they represent the major contributor of inflammasome-dependent inflammatory activity in the CNS (Halle *et al.*, 2008; Sarkar *et al.*, 2017a; Scheiblich *et al.*, 2017). Astroglia have also an extensively characterized role in inflammation during neurodegeneration, injury, and repair. The role of astroglia in the inflammatory response in PD is complex and manifests as either neuroprotective or detrimental depending on the trigger, timing, and anatomic context of the response. Whether astroglia express functional inflammasomes remains the subject of debate. Whereas studies have indicated inflammasome activity in astroglia (Johann *et al.*, 2015), other studies focused on *Nlrp3* have concluded that astrocytes do not express functional inflammasomes (Gustin *et al.*, 2015; Martinez *et al.*, 2017). Oftentimes, studies have been conducted in primary mixed glial cultures where the isolation of astrocytes without microglial contamination remains a technical challenge. What is clear is that astroglia respond to microglia-derived stimulus including IL-1B (Moynagh, 2005) and participate in the inflammatory response in the nigrostriatal system in PD (Booth *et al.*, 2017). In addition to glia, recent evidence indicates that other CNS lineages may also utilize the inflammasome danger-sensing platform. Neurons are capable of directly modulating the inflammatory response through the release of electrochemicals and neuropeptides in a process described as neurogenic inflammation (Richardson and Vasko, 2002). Whereas the function of inflammasomes in neurogenic inflammation is poorly understood, expression of the *NLRP1* (de Rivero Vaccari *et al.*, 2008), *AIM2* (Adamczak *et al.*, 2014), and *NLRP3* (Zhang *et al.*, 2016) NLRs has been detected in neurons, with our laboratory recently reporting elevated NLRP3 expression in pigmented

neurons of the PD mesencephalon (von Herrmann *et al.*, 2018). These findings suggest that inflammasomes may function in neurons providing a novel mechanism of neurogenic inflammation capable of initiating or influencing neuroinflammation through a mechanism potentially culminating in pyroptotic cell death. In addition, peripheral monocytes exhibiting canonical inflammasome activity enter and exit the CNS microenvironment monitoring the state of CNS tissues during the neurodegenerative processes occurring in PD (Grozdanov *et al.*, 2014) and cerebral endothelial cells are perturbed in PD and express multiple NLRs, along with caspase-1 and IL-1B whose expression can be induced using LPS (Nagyőszi *et al.*, 2015). In summary, whereas microglia are clearly a major source of inflammasome activity in the CNS, emerging evidence suggests that these cytosolic danger sensors may function in multiple cell types. Given that inflammasome-competent cells exist throughout the CNS, and at multiple interfaces between the CNS and the environment, continued experiments to understand whether inflammasomes function as a core environmental stress response pathway in the CNS are warranted.

INFLAMMASOMES IN PARKINSON'S DISEASE

The evidence for neuroinflammation occurring in association with PD is well-established and has been extensively reviewed by others (Hirsch *et al.*, 2012; Tansey and Goldberg, 2010). In PD, sterile cellular stress can manifest in several ways including metabolic and oxidative stress (Bosco *et al.*, 2006), dysregulation of autophagy and prion-like propagation of misfolded proteins (Luk *et al.*, 2012; Mao *et al.*, 2016), and the accumulation of toxic DA metabolites (Burbulla *et al.*, 2017). Here, we discuss evidence supporting a role for inflammasomes in the response to these PD-associated manifestations of cellular stress that provides mechanistic clues as to how toxicant exposure may interact with and exacerbate these cellular pathologies thereby impacting PD progression. The activity of inflammasomes has been linked to synuclein pathology, widely believed to be modulated by environmental toxicant exposure (Liu and Yang, 2005; Pan-Montojo *et al.*, 2012; Villar-Piqué *et al.*, 2016). Monocytes isolated from healthy donors are capable of phagocytosing fibrillary α-synuclein, and pathologic α-synuclein multimers more robustly induce NLRP3-associated caspase-1-dependent IL-1B maturation as compared with α-synuclein in monomeric form (Codolo *et al.*, 2013). It is important to note that these studies in monocytes are conducted in the context of LPS exposure, a bacterial endotoxin that is sufficient to induce a robust IL-1B response in monocytes without requiring an additional inflammasome activation step. It remains to be seen whether α-synuclein can act as an inflammasome stimulus in microglia under sterile conditions. In cultured neuroblastoma cells, activation of the inflammasome results in caspase-1-dependent cleavage of α-synuclein. Caspase-1 truncation of α-synuclein increased protein aggregation and could be inhibited using small molecule caspase-1 inhibitors (Wang *et al.*, 2016). In a related study, inhibition of caspase-1 using the brain permeant pro-drug VX-765 mitigated synucleinopathy and nigral cell loss in a mouse model of multiple system atrophy (Bassil *et al.*, 2016). Many environmental toxicants target mitochondrial pathways and mitochondrial dysfunction is well-characterized in idiopathic PD (Langston *et al.*, 1983) with numerous genes associated with hereditary PD also impacting mitochondrial homeostasis and the cellular redox state (Vives-Bauza *et al.*, 2010). The PINK1/Parkin pathway is central to mitochondrial quality control (for review [Pickrell and Youle, 2015]) and inactivation of either of these genes causes rare hereditary forms of PD (Kitada *et al.*, 1998; Valente *et al.*, 2004). The NLRP3 inflammasome relies on mitochondria for assembly and function and mitochondrial deregulation can result in inflammasome activation (Elliott *et al.*, 2018; Sadatomi *et al.*, 2017; Zhou *et al.*, 2011). Deregulation of PINK1/Parkin-mediated mitophagy results in the accumulation of impaired mitochondria (Geisler *et al.*, 2010; Matsuda *et al.*, 2010). Loss of PINK1/Parkin function exacerbates Nlrp3 inflammasome activity in primary microglia in a manner that can be mitigated by the small molecule Nlrp3 inflammasome inhibitor MCC950 (Mouton-Liger *et al.*, 2018) consistent with findings that impaired mitophagy and subsequent generation of ROS activated the NLRP3 inflammasome in bone marrow-derived macrophages (Zhou *et al.*, 2011). Mutations in LRRK2 are prevalent in PD having been identified in both idiopathic and autosomal dominant disease. PD-associated mutations in LRRK2 result in loss-of-function which has been subsequently associated with elevated oxidative stress (Angeles *et al.*, 2011) and mitochondrial fragmentation (Wang *et al.*, 2012). In *Lrrk2*$^{-/-}$ macrophages, an Lrrk2/Nlrc4 interaction is disrupted resulting in reduced phosphorylation of Nlrc4, impaired inflammasome activation, and deficiencies in the host-pathogen response (Liu *et al.*, 2017). Dopamine can modulate the immune response (Besser *et al.*, 2005; Farber *et al.*, 2005) and recent reports indicate that dopamine regulates the activity of the Nlrp3 inflammasome in bone marrow-derived macrophages and that this function relies on the presence of the DRD1 receptor (Yan *et al.*, 2015). In the same report, *Nlrp3*$^{-/-}$ mice resisted MPTP-mediated nigral degeneration whereas similarly treated *Drd1*$^{-/-}$ mice were more sensitive to the toxin and had enhanced inflammasome activity. These findings provide strong support for the authors conclusion that dopamine functions to inhibit inflammasome activity and that suppression of Nlrp3-driven neuroinflammation may be neuroprotective for PD. Dopamine metabolism results in the generation of toxic by-products that are posited to underlie dopamine neuron susceptibility in PD (Burbulla *et al.*, 2017). In addition to increasing oxidative stress, dopamine metabolism by-products accumulate in the form of neuromelanin granules reminiscent of the crystalline activators of inflammasomes observable in inflammatory disorders such as gout (Yagnik *et al.*, 2000). Although research in inflammasomes and dopamine neuron susceptibility is in its early stages, our recent report (von Herrmann *et al.*, 2018), and the potential that CNS permeant environmental toxicants may enter neurons suggests that dopamine neurons may be a cell type of interest in the initiation and propagation of inflammasome-driven neuroinflammation in PD. Taken together, it is noteworthy that well-characterized aspects of PD-related cell stress and death either have already been linked to inflammasome activity or are readily predicted based on the known mechanisms of inflammasome activation as identified in other systems. Whereas not unique to PD, the overwhelming evidence of an environmental component to the disease has prompted intense study to determine how potentially deleterious exposures can be detected and prevented. In the following section, we describe evidence supporting a role for inflammasomes at the intersection between environmental toxicants and aforementioned cellular pathologies, so well characterized in PD.

INFLAMMASOMES IN THE CELLULAR RESPONSE TO PARKINSON'S-ASSOCIATED TOXICANTS

Pesticides

Pesticide exposure has been identified as a risk factor for the development of PD (Costello *et al.*, 2009; Hatcher *et al.*, 2008;

Liou *et al.*, 1997; Tanner *et al.*, 2011; Wang *et al.*, 2014) and multiple brain-permeant pesticides have been either directly linked with inflammasome activation or have been characterized as invoking cellular stress known to be associated with inflammasome activation. Among the best characterized in the context of inflammasome activation is rotenone, a broad-spectrum organic pesticide used in agriculture and to cull unwanted fish populations in water management projects (Krumholz, 1948). Rotenone is lipophilic, easily crossing the BBB (Figure 2), and exposure has been associated with the development of PD in agricultural workers [7]. Chronic exposure in rodents recapitulates some of the symptomology of PD, resulting in nigrostriatal degeneration, the accumulation of α-synuclein positive inclusions, motor impairment, and neuroinflammation (Cannon *et al.*, 2009; Martinez *et al.*, 2017). Rotenone toxicity results from inhibition of mitochondrial complex I, implicating rotenone as a potentiator of mito-ROS-mediated inflammasome activation (Figure 3) (Sherer *et al.*, 2003). In support of this premise, the Nlrp3 inflammasome responds to mitochondrial damage, ROS, and proteinopathy and rotenone activates the Nlrp3 inflammasome within both peripheral and CNS-derived cells *in vitro* (Lawana *et al.*, 2017; Won *et al.*, 2015). In mice, *Nlrp3* loss prevents the degeneration of nigral dopaminergic neurons resulting from chronic rotenone exposure (Martinez *et al.*, 2017). Microglial inflammasome activity has been associated with rotenone-based neurodegeneration *in vitro* where studies indicate that the amplification of Nlrp3 signaling caused by rotenone-dependent mitochondrial impairment augments dopaminergic neuron degeneration (Sarkar *et al.*, 2017b).

Whereas rotenone-mediated inflammasome activation has been most widely studied, evidence suggests that inflammasomes may respond similarly to other PD-associated pesticides. Paraquat is a widely used, non-selective, contact-based herbicide also linked to an increased risk of PD (Tanner *et al.*, 2011). Differing from rotenone, paraquat's chemical structure is similar to that of MPP^+, the causal metabolite of the PD-associated drug-of-abuse MPTP (Wang, Ren, *et al.*, 2017). Initially, paraquat was thought to be unable to cross the BBB (Naylor *et al.*, 1995) but more recent studies suggest that it can in fact bypass the BBB and directly enter the brain (Figure 2) (Shimizu *et al.*, 2001, 2003). Once in the CNS, paraquat can be reduced to the monovalent cation PQ^+ by NADPH oxidase in microglia and enter other CNS cells including neurons by co-opting the dopamine transporter, organic cation transporter 3, and neutral amino acid transporters (Shimizu *et al.*, 2003). Systemic administration of paraquat results in degeneration of dopaminergic neurons of the substantia nigra pars compacta (Manning-Boğ *et al.*, 2003; McCormack *et al.*, 2002; Ossowska *et al.*, 2006) and the combination of paraquat and maneb is widely used to model PD in rodents (Cicchetti *et al.*, 2005). Direct evidence of inflammasome activity in response to paraquat in the CNS is limited. However, paraquat exposure in animal models elicits inflammasome-related cellular stressors including mitochondrial ROS (McCarthy *et al.*, 2004) and α-synuclein aggregation (Manning-Bog *et al.*, 2002) (Figure 3). Among these pathologies, evidence has emerged to support a prediction that mito-ROS-based inflammasome activation may occur in paraquat-based models of PD. In support of this prediction, detoxification of mitochondrial ROS suppressed NLRP3 activation and the inflammatory response in a paraquat-based mouse model of Alzheimer's disease (Chen *et al.*, 2015). Paraquat-induced cytotoxicity, ROS generation, and resultant NLRP3 activation were reduced in monocytes pre-treated with the anti-oxidant silymarin (Liu *et al.*, 2018). Indicating a potential role for inflammasomes in paraquat-driven dopaminergic neurodegeneration, nigral cell loss resulting from paraquat exposure is enhanced by an endotoxin priming step highly suggestive of the canonical two-step model of inflammasome activation (Purisai *et al.*, 2007) (Figure 1). Although direct characterization of the mechanism by which paraquat may interact with inflammasomes in PD has not been completed, the currently available database provides ample support for the pursuit of such studies.

Mechanistic data related to the organophosphate chlorpyrifos (CPF) is limited, but this pesticide bears mention because it is widely used, entering the environment at an estimated 3.2–4.1 million kilograms annually in the U.S. alone (Solomon *et al.*, 2014), and persists in soil and water (Mackay *et al.*, 2014). Epidemiological studies have found a link between CPF exposure and an increased risk of PD (Dhillon *et al.*, 2008; Gatto *et al.*, 2009; Manthripragada *et al.*, 2010). Parran and others reported that CPF and its metabolites were able to cross a membrane co-culture of bovine microvascular endothelial cells and neonatal rat astrocytes occurring in association with a concentration-dependent decrease in electrical resistance (Parran *et al.*, 2005) (Figure 2). Supporting this finding, CPF transiently altered the expression of proteins important for BBB integrity, such as claudin5, ZO1, and TRPC4 (Li and Ehrich, 2013) and following systemic administration in mice, CPF and its metabolites are identifiable within CNS tissues (Kopjar *et al.*, 2018; Williamson Leah *et al.*, 2006). CPF and its metabolites act through the inhibition of acetylcholinesterase (AchE) (Colombo *et al.*, 2005) and CPF-induced neuronal stress and apoptosis have been identified in a variety of models including zebrafish (Garcia-Reyero *et al.*, 2016), rat primary cortical neurons (Caughlan *et al.*, 2004), and the human catecholaminergic neuroblastoma cell line SH-SY5Y (Eun *et al.*, 2014; Ki *et al.*, 2013; Raszewski *et al.*, 2015). Experimental evidence of neurodegeneration resulting from chronic CPF exposure is limited. However, dopaminergic cell loss was observed in CPF-exposed neonatal rats (Zhang *et al.*, 2015). Suggestive of a role for inflammasomes in the response to CPF, studies identify oxidative stress within the CNS (Figure 3) and CNS-derived cell lines (Verma *et al.*, 2007) and in oligodendrocyte progenitors upon CPF exposure (Saulsbury *et al.*, 2009). In human keratinoctyes, CPF-induced ROS was associated with elevated levels of NLRP3 and subsequent apoptosis (Jang *et al.*, 2015). Further studies need to be done to characterize the effect of CPF exposure on inflammasome activation in the context of the CNS.

Heavy Metals

In addition to pesticides, heavy metal toxicants have been linked with the development of parkinsonism (for review [Caudle *et al.*, 2012]). Manganese (Mn) is an essential nutrient but it is also released into the environment through industrial activities and has been widely studied in the context of PD and inflammasome activation. In both the occupational and laboratory setting, Mn exposure has been implicated in the development of inflammation (Kresovich *et al.*, 2018; Santos *et al.*, 2013), manganism, and progressive parkinsonism (Racette *et al.*, 2017). Mn-associated PD symptomology is distinct from idiopathic PD (Olanow, 2004), however, evidence indicates that the dopamine system is impacted by Mn exposure in non-human primates (Guilarte *et al.*, 2008) and rodent models (Sanchez-Betancourt *et al.*, 2012; Zhao *et al.*, 2009) making the understanding of Mn toxicity highly relevant to PD and similar disorders. Mn crosses the BBB as Mn^{2+}, in complex with transferrin, or in complex with citrate (Figure 2). Each of these forms enters the brain via a store-operated calcium channel (SOCC). Passage into the brain

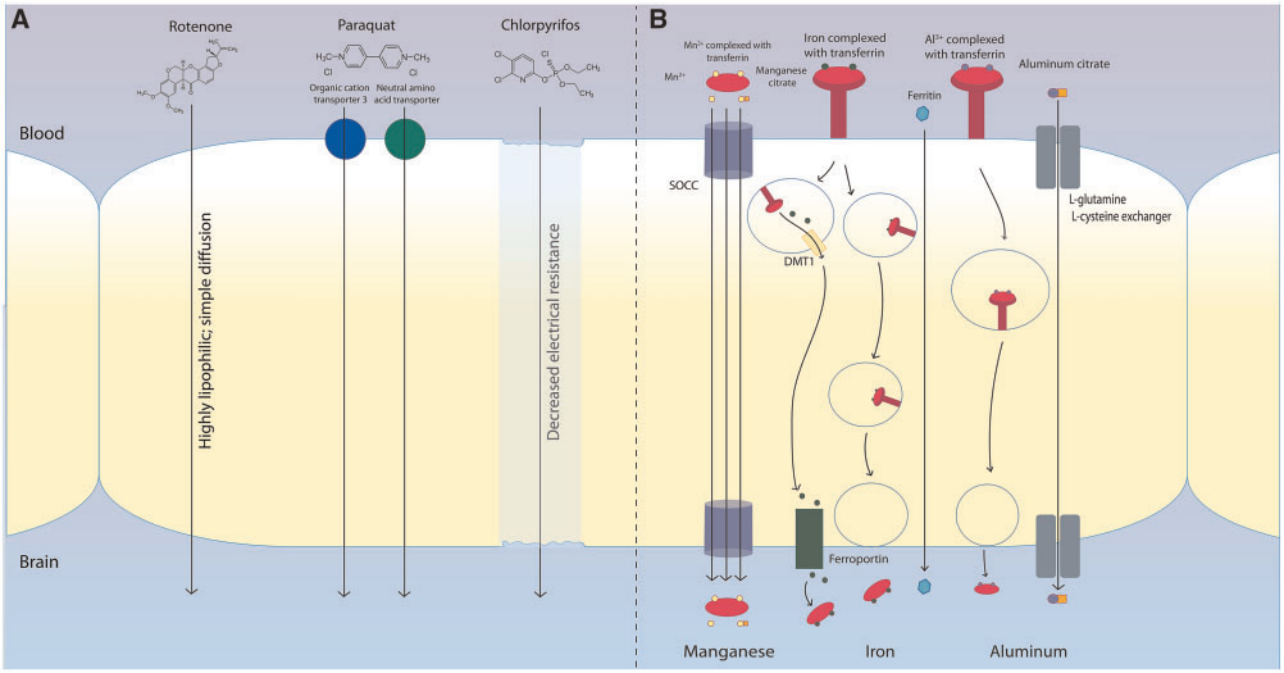

Figure 2. Mechanisms of toxicant transportation across the blood-brain barrier. Many environmental toxicants associated with inflammasome activity are able to bypass the blood-brain barrier (BBB). The pesticide rotenone is highly lipophilic, easily crossing the BBB through simple, passive diffusion. The herbicide paraquat enters the brain through neutral amino acid transporters. The organophosphate pesticide chlorpyrifos crosses the BBB by altering electrical resistance resulting in decreased membrane integrity. The heavy metal manganese has been shown to cross the BBB as the Mn^{2+} ion alone or in complex with transferrin or citrate. Each of these three forms crosses the BBB through a store-operated calcium channel (SOCC). Iron crosses the BBB through three different methods: endocytosis of ferritin and its receptor coupled by a release into the cytoplasm by DMT1 and transport into the brain through ferroportin, endocytosis of ferritin and its receptor for release into the brain, or direct transport of ferritin across the BBB. Aluminum crosses the BBB largely in complex with transferrin or citrate, likely through the L-glutamine, L-cysteine exchanger.

via the SOCC has been shown to be more rapid than simple diffusion from the brain, the primary method of exit, likely underlying the observation of Mn accumulation in brain tissue (Antonini *et al.*, 2009; Jenkitkasemwong *et al.*, 2018; Uchino *et al.*, 2007; Yokel, 2009). Several lines of evidence support indirectly a role for microglial inflammasomes in Mn toxicity. Mn accumulation results in microglial activation and exacerbates LPS-mediated IL-1B production, suggestive of two-step inflammasome activation (Filipov *et al.*, 2005). Mechanistically, evidence from MitoPark mice indicates that Mn exposure can exacerbate mitochondrial stress suggesting the potential for mito-ROS-mediated inflammasome activation (Langley *et al.*, 2018) (Figure 3). Beyond circumstantial evidence of Mn-induced mito-ROS-mediated inflammasome activation, subacute Mn exposure resulted in microglial NLRP3 inflammasome activation associated with dysregulation of autophagy and lysosomal stress (Wang, Zhang, *et al.*, 2017). Authors conclude that Mn-driven assembly of the autophagosome and elevated cathepsin B are required for Nlrp3 inflammasome activation supporting a role for the lysosomal rupture model of inflammasome activation and reminiscent of the related stress outcomes associated with proteinopathy.

Other PD-implicated heavy metals may also interact with the inflammasomes. Iron deposition in the substantia nigra has been long recognized (Hallgren and Sourander, 1958), is considered a potential imaging biomarker of PD (Bartzokis *et al.*, 1999), and nigral iron levels are correlated with PD severity (Martin-Bastida *et al.*, 2017). Mechanisms for iron to cross the BBB include direct transport of ferritin across the barrier and endocytosis of transferrin and its receptor for release into the brain. Once iron enters the CNS it can be stored in ferritin or released via ferroportin (Duck *et al.*, 2017) (Figure 2). Rodent models of PD indicate that iron has a broad role in increasing the permeability of the BBB suggesting the potential for a more generalizable role for iron in the exacerbation of neuroinflammation and toxicity (Olmedo-Diaz *et al.*, 2017). Monocytes traffic across the BBB into the CNS (Depboylu *et al.*, 2012) and have an underappreciated potential for driving both systemic and neuroinflammation in PD (Grozdanov *et al.*, 2014). Monocytes play a key role in iron homeostasis and recent evidence indicates the labile iron activates the Nlrp3 inflammasome through a ROS-dependent mechanism (Nakamura *et al.*, 2016). Such an activity supports an intriguing model in which inflammasome activity in monocytes may provide insight into inflammatory processes in the CNS detectable in the periphery. Direct evidence is sparse but studies indicate that iron-mediated inflammasome activation may also occur in CNS microglia (Figure 3). Microglia function in iron homeostasis (Rathore *et al.*, 2012) and rodent and human studies identify a correlation between microglial activation and increased iron levels (Olmedo-Diaz *et al.*, 2017). Mito-ROS-mediated inflammasome activation is likely given that microglia are hypothesized to produce ROS whereas simultaneously promoting iron uptake. Over time, the ROS generated leads to release of stored iron in the microglia, which can further increase ROS production (Garrido-Gil *et al.*, 2013) and impact neuronal iron homeostasis and death (Wang *et al.*, 2013; Zhang *et al.*, 2014).

Studies of aluminum (Al) toxicity in PD are limited, but noteworthy, due to potential associations between Al exposure and related neurodegenerative disorders (Mirza *et al.*, 2017). Al enters the brain in a similar mechanism to iron with 90% of Al complexed with transferrin and endocytosed at the BBB, whereas the remainder is complexed with citrate and likely taken up by a L-glutamine/L-cysteine exchanger (Nagasawa

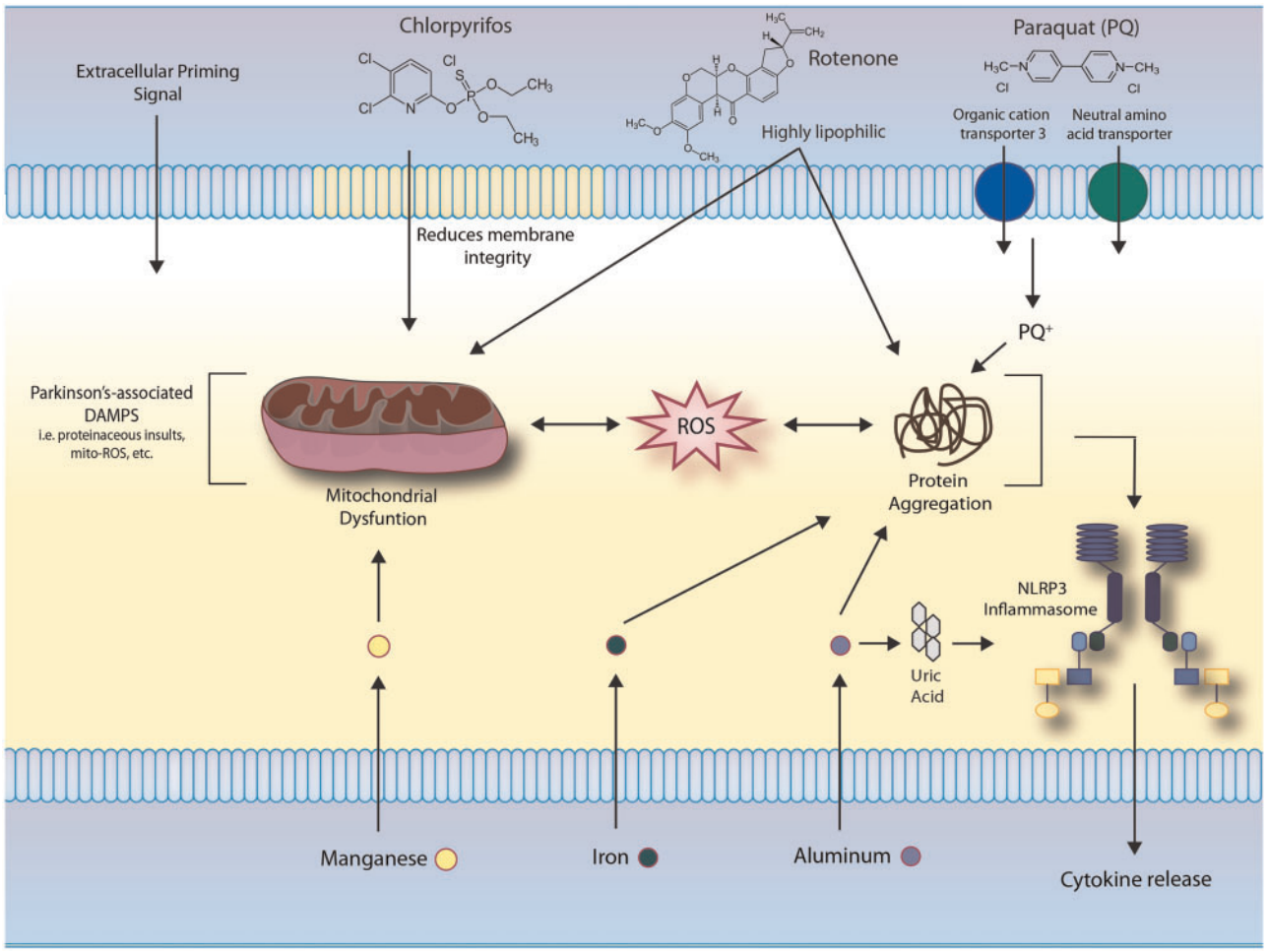

Figure 3. Environmental toxicants exacerbate Parkinson's-associated sterile molecular patterns associated with activation the NLRP3 inflammasome. Parkinson's-associated cytopathologies, such as the aggregation of misfolded proteins and generation of mitochondrial ROS, can stimulate inflammasome activity. Rotenone inhibits mitochondrial complex I decreasing synthesis of ATP and deregulating electron transfer, leading to generation of ROS. Paraquat exposure elicits inflammasome-related cellular stressors such as α-synuclein protein aggregation. Chlorpyrifos exposure elevates ROS production in association with mitochondrial dysfunction. IL-1B cytokine release is increased as a result of manganese accumulation in LPS-primed glial cells, consistent with canonical two-step inflammasome activation. In addition, manganese exposure increases mitochondrial and oxidative stress. Buildup of labile iron results in protein aggregation. Free ferrous iron can react to produce ROS, resulting in activation of the NLRP3 inflammasome. Aluminum exposure also propagates the misfolding of proteins, including pathologic α-synuclein, and contributes to ROS production and the formation of uric acid, all of which can activate the NLRP3 inflammasome.

et al., 2005) (Figure 2). Similar to iron, mito-ROS-mediated inflammasome activation appears most likely. In a comparative study that included multiple common metals, Al exposure resulted in the most robust induction of ROS in glial cells (Pogue *et al.*, 2012). Interestingly, Al exposure may also be related to other potential inflammasome triggers, including pathologic α-synuclein, as observed in an *in vitro* neuronal cell model (Saberzadeh *et al.*, 2016). Perhaps the most indicative of Al's potential to activate the inflammasomes, Al exposure has been demonstrated to generate uric acid in exposed tissues, a well-known activator of the NLRP3 inflammasome (Kool *et al.*, 2008) (Figure 3).

FUTURE DIRECTIONS

The study of inflammasomes in PD follows extensive characterization of these intracellular mediators in the context of innate immunity. The recent interest in evaluating inflammasomes in PD stems from important and longstanding questions surrounding the cellular mechanisms underlying neuroinflammation and environmental risk, two well-known but incompletely understood aspects of PD. Fueling excitement in exploring the inflammasomes in PD is the potential that these pathways may be broadly employed mechanisms linking environmental insult with multiple aspects of PD-associated cellular stress. Moreover, inflammasomes are enzymatically active multi-protein complexes that can be modulated pharmacologically. Based on the accumulating data reviewed herein, the interest in inflammasomes in PD is certainly warranted, however, many open questions remain related to the functioning of these danger-sensing platforms in the CNS.

Core pathologies evident in PD, namely mitochondrial stress, proteinopathy, and cell death, are intuitive modulators of inflammasome activity and it is likely that the ongoing evaluation of inflammasomes in the context of these pathologies will continue to reveal new roles for inflammasomes in PD. One open question surrounds the concept of a 'two-hit' hypothesis for inflammasome activation. In many model systems, an initial priming trigger likely operating through toll-like receptors

(TLRs) upregulates the components and targets of the inflammasomes and a subsequent stimulus triggers the catalytic events resulting in cytokine maturation and possible pyroptosis. Whether such a priming step is required for PD-associated inflammasome activity is unclear. One possibility is that indeed a pathogenic insult may prime the CNS for inflammasome activation. In support of this model, viral infections have been posited as having a role in the etiology of PD (Jang *et al.*, 2009). Alternatively, in the absence of pathogenic priming, neuronal death could produce both the priming and activating stimuli based on the release of intracellular contents including pathologic α-synuclein, known to activate microglial TLR4 (Fellner *et al.*, 2013), along with other canonical inflammasome stimulators such as ATP (Schroder and Tschopp, 2010). These findings are important because they begin to delineate the means by which the neurodegenerative microenvironment may engage canonical inflammasome activation through sterile mechanisms. However, it is likely that additional non-canonical inflammasome activation mechanisms also exist. As noted in previous sections, inroads toward understanding non-canonical inflammasome signaling in PD have been made in monocytes where synuclein priming is sufficient for assembly of the intracellular inflammasome in the absence of an activating stimulus (Codolo *et al.*, 2013). Intriguingly, aging itself has been posited as a priming factor (Bauernfeind *et al.*, 2016). Youm and others report that inactivation of *Nlrp3* suppresses the development of age-related neuroinflammatory changes and functional decline in mice (Youm *et al.*, 2013). Looking forward, we are in the early stages of understanding how inflammasomes may impact age-related neurologic disorders and how these processes may be exacerbated by environmental toxicants.

Pyroptosis is an intriguing area for exploration in the CNS, particularly in the context of cytotoxic inflammatory toxicants. Unlike classical apoptosis, pyroptotic cell death is pro-inflammatory, characterized by the formation of pores in the cytoplasmic membrane, cellular lysis, and significant release of intracellular material [19]. Such a mechanism is intriguing and suggests that inflammasome activation in CNS cells leading to pyroptosis may not only continually exacerbate co-occurring neuroinflammation throughout the disease course, but could also result in the release of CNS-specific biomolecules into the interstitium and possibly into systemic circulation. Although we do not fully understand how such a process could be initiated and unfold in the context of toxicant exposure in PD, evidence of neurologic disease-associated intracellular proteins in the cerebrospinal fluid (Irwin *et al.*, 2018; Wang *et al.*, 2018) and plasma (Lin *et al.*, 2017) provide a strong rationale for further investigation of the pyroptotic process. Going forward, systemic analysis of inflammasome proteins including caspase-1 and IL-1B along with other indicators such as cleaved gasdermin D, recently discovered to be required to drive pyroptosis and associated IL-1B release (Liu *et al.*, 2016), will be of great interest. Although initially characterized in macrophages (Brennan and Cookson, 2000), this concept is now supported by evidence of pyroptosis in the CNS, with hippocampal neurons (Fann *et al.*, 2013) and retinal photoreceptor cells (Viringipurampeer *et al.*, 2016) demonstrated to undergo NLRP3-dependent pyroptosis. Findings from ongoing studies in this area are awaited with great anticipation because they have the potential to identify not only novel intracellular mechanisms of cell death associated with environmental toxicants and PD, but may also reveal unexpected biologic indicators of ongoing neurodegenerative and neuroinflammatory processes relevant to a panoply of neurologic disorders.

A key finding in our review of current literature was the preponderance of studies focused on the NLRs; particularly NLRP3. In these early stages, evaluation of NLRP3 is appropriate based on its recognition as a mediator of sterile inflammation, as compared with other inflammasome-related NLRs whose activities are primarily characterized in the response to biological toxins and intracellular pathogens. Based on unexpected findings from studies of NLRP3 in the CNS, it stands to reason that continued analyses of all inflammasome proteins with an eye toward the identification of non-canonical signaling mechanisms will identify novel pathways of interest with appreciable impact on our understanding of health and disease. Another key aspect of the inflammasomes is their potential as therapeutic targets. These cytosolic multi-protein complexes rely on cell surface receptors, inducible protein interactions, and catalytic events whose interruption with small molecules is already under intense evaluation as noted by many recent reviews (Lopez-Castejon and Pelegrin, 2012; Ozaki *et al.*, 2015). These translational efforts will be catapulted forward by further characterization of the basic mechanisms of inflammasome activity and the identification of new protein interactions, biologic indicators of inflammasome activity, and the development of high throughput methods for screening potential anti-inflammasome compounds. Given the growing body of evidence that modulation of inflammasomes may impact the progression of human diseases and the relative manageability of side-effects associated with anti-inflammatories, anti-inflammasome drugs have a reasonable potential for utility in the clinic, especially in age-related, slowly progressive disorders like PD.

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