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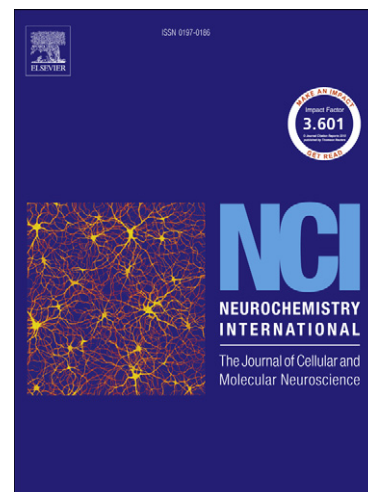
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Neuroinflammation and oxidative stress: co-conspirators in the pathology of Parkinson's disease.

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Abbreviations:

6-OHDA, 6-hydroxy dopamine; AR-PD; autosomal recessive Parkinson's disease; BBB, blood-brain barrier; COX, cyclo-oxygenase; CR3/43, MHC Class II; DA; dopaminergic/dopamine; DAMPS, Damage-associated molecular patterns; EBM11, anti CD-68; EGF, epidermal growth factor; FGF, fibroblast growth factor; GDNF, glial derived neurotrophic factor; GSH, glutathione; GPx, Glutathione peroxidase; 4-HNE, 4-hydroxynonenal; IFN, interferon; ICAM-1, Intercellular Adhesion Molecule 1; IL-, interleukin; LFA-1, Lymphocyte function-associated antigen 1; LPS, Lipopolysaccharide; LRRK2, Leucine-rich repeat kinase-2; MHC, Major histocompatibility complex; MPTP, 1-methyl-4-phenyl-1, 2, 3, 6-tetrahydropyridine; NO, nitric oxide; NOS, nitric oxide synthase; NSAIDS, non-steroidal anti-

inflammatory drugs; PAMP, Pathogen-associated molecular patterns; PD, Parkinson's disease; PINK1, PTEN-induced putative kinase-1; PRR, pattern recognition receptor; ROS, reactive oxygen species; SN, substantia nigra; SOD, superoxide dismutase; TGF, transforming growth factor; TLR, Toll-like receptor; TNF, tumour necrosis factor

ABSTRACT

Parkinson's disease (PD) is a complex disease, with genetics and environment contributing to the disease onset. Recent studies of causative PD genes have confirmed the involvement of cellular mechanisms engaged in mitochondrial and UPS dysfunction, oxidative stress and apoptosis in the progressive degeneration of the dopaminergic neurons in PD. In addition, clinical, epidemiological and experimental evidence has implicated neuroinflammation in the disease progression. This review will discuss neuroinflammation in PD, with particular focus on the genetic and toxin-based models of the disease. These studies have confirmed elevated oxidative stress and the pro-inflammatory response occurs early in the disease and these processes contribute to and/or exacerbate the nigro-striatal degeneration. In addition, the experimental models discussed here have also provided strong evidence that these pathways are an important link between the familial and sporadic causes of PD. The potential application of anti-inflammatory interventions in limiting the dopaminergic neuronal cell death in these models is discussed with evidence suggesting that the further investigation of their use as part of multi-targeted clinical trials is warranted.

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1. INTRODUCTION

It has been over 50 years since the seminal observation from Arvid Carlsson that dopamine is used in the central nervous system as a neurotransmitter (Carlsson et al., 1957). During this time the death of dopaminergic nerves have been implicated as the central cause for the development of Parkinson's disease (PD). PD is the second most common neurodegenerative disorder worldwide, with a prevalence of approximately 1% in people over age 60 and rising to over 4% by age 85 (de Lau et al., 2006). However, an estimated 3% of cases are identified in individuals younger than age 50 (Hawkes, 2008). Clinically, the disease is characterised by resting tremor, rigidity, bradykinesia and postural instability (Jankovic et al., 2007; Smeyne et al., 2005). The development of these classical motor symptoms can be attributed to the selective loss of dopaminergic (DA) neurons, primarily in the substantia nigra pars compacta (SN). This hallmark loss of neurons results in striatal dopamine depletion and a resultant dysfunction of the basal ganglia, a cluster of nuclei involved in the initiation and execution of movement (Rodriguez-Oroz et al., 2009). Pathologically, the disease is also characterised by the presence of proteinaceous cytoplasmic inclusions known as Lewy bodies (Lees et al., 2009). Although motor symptoms remain the major criteria for clinical diagnosis, non-motor symptoms including impaired olfaction, constipation, sleep disorders and various neuropsychiatric manifestations can become prominent both before and during PD onset and progression (Chaudhuri et al., 2009).

To date, no effective therapies have been developed to cure PD; however, pharmaceutical treatments focusing on relief and management of symptoms are available. In the early stages of PD, motor symptoms respond well to dopamine replacement therapy, achieved by the administration of L-DOPA, a precursor in the synthesis of dopamine (Birkmayer et al., 1962). The fact that a therapy first used in the 1960s remains the front line treatment choice for PD underscores our lack of understanding of the underlying causes of neuronal degeneration in this disease. This review will focus on the molecular mechanisms and interplay by which

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neuroinflammation and oxidative stress influence the aetiology of PD, with the aim to identify the molecular pathways that can be targeted to retard the progression of the DA cell death in PD.

2. AETIOLOGY OF PD

PD is considered to be a multi-factorial disorder with both genetic defects and exposure to environmental factors influencing disease progression (reviewed in Gao *et al.*, 2011). The majority of PD cases are sporadic and idiopathic with only 10% of all cases linked to a genetic cause. To date, 16 PARK loci have been reported, with *SNCA* (PARK1/4) and *LRRK2* (PARK8) and *parkin* (PARK2) and *PINK1* (PARK6) being the most common causes of autosomal dominant (AD-PD) and autosomal-recessive (AR-PD) PD, respectively (reviewed in (Martin et al., 2011)). In addition, mutations in *parkin, SNCA and LRRK2* have also been reported in sporadic PD, suggesting an influence over progression and age of onset of the disease (reviewed in (Kumari et al., 2009; Moore et al., 2005). Monogenic and sporadic forms of PD display distinct clinical and pathological features. However, there are many overlapping features, specifically the nigral degeneration, advocating common disease mechanisms. Animal models of the disease support a critical interaction between genetics and the environment in PD pathogenesis. While genetic models of PD generally do not display any overt phenotype (loss of DA neurons or the presence of Lewy bodies), two-hit models involving the use of environmental toxins in these animals are often a better representation of the human pathology. These combined models are useful tools for understanding the underlying mechanisms leading to the neurodegeneration in PD. Indeed, alterations in protein processing, mitochondrial dysfunction and elevated oxidative stress have all been linked to the disease pathogenesis (Obeso et al., 2010). In addition, it is now well accepted that chronic neuroinflammation is pathological feature of the disease (Hirsch et al., 2009; Lee et al., 2009) **(Figure 1)**.

3. NEUROINFLAMMATION

The immune responses within the brain are tightly regulated, a phenomenon first described by Sir Peter Medawar as "immune privilege" (Medawar, 1948). This regulation is partly dependent on the blood-brain barrier (BBB) but also the modulation of the response by the resident cells of the brain, the microglia and astrocytes (and to a lesser extent, neurons). Microglia are the major resident immune cells in the brain, providing innate immunity, however astrocytes and oligodendrocytes are also involved in the neuroinflammatory response (Tansey et al., 2007). Microglia maintain the homeostasis of the brain through the production of various neurotrophic (Brain-Derived Neurotrophic Factor (BDNF), Insulin-like Growth Factor-1 (IGF-1)) and anti-inflammatory factors (IL-10), influencing surrounding astrocytes and neurons. In the healthy brain, the resident microglia exhibit a resting phenotype. However, under stresses such as pathogen invasion, injury, or toxic protein accumulation, microglia become activated, initiating immune responses to instigate tissue repair by clearing debris and apoptotic cells and by releasing growth factors (reviewed in (Aloisi, 1999)). If controlled, the innate immune response will resolve once the initial stress has been eradicated. However, persistence or a failure in the resolution of the inflammatory stimuli will lead to the overproduction of neurotoxic factors including cytokines, chemokines, and prostaglandins. Pro-inflammatory cytokines such as Tumour Necrosis Factor-α (TNF-α) and Interleukin-1β (IL-1β) act on astrocytes, inducing the adaptive immune response while chemokines such as monocyte chemotactic protein-1 (MCP-1)/Chemokine (C-C motif) ligand 2 (CCL2) recruit additional immune cells. In addition, these inflammatory responses may potentiate neuronal cell damage through the generation of reactive oxygen and nitrogen species (ROS/RNS). The inflammatory response in the brain involves both the innate and adaptive immune systems to provide a balance between beneficial and deleterious effects.

3.1 Innate Immune Response

CNS cells can mount innate immune responses through conserved pattern-recognition receptors (PRRs). The Toll-like receptors (TLRs) bind highly conserved structural motifs either from pathogens (pathogen associated molecular patterns; PAMPs) or from damaged or stressed cells (damaged-associated molecular patterns; DAMPs). Therefore, not only can invading micro-organisms (bacteria, viruses) activate an innate immune response in the CNS, evidence suggests endogenous signals such as heat shock proteins, high mobility groups box chromosomal protein 1 (HMGB-1), DNA, Aβ, α-synuclein and tau can also (Arroyo et al., 2011). TLR expression is upregulated in a wide range of CNS disorders in microglia, astrocytes and neurons and is generally associated with increased production of pro-inflammatory cytokines to promote neuronal cell death (Carty et al., 2011). The nucleotide-binding oligomerization domain-like receptors (NLRs) are another family of PRRs expressed in both microglia and astrocytes that recognize invading pathogens to initiate a pro-inflammatory response (Chauhan et al., 2009). The receptor for advanced glycation end-products (RAGE) has also been implicated in the release of pro-inflammatory cytokine and free radicals in neurological disorders through the ligand, HMGB-1. It is expressed on both neurons and astrocytes and is increased following oxidative stress and immune/inflammatory responses, thereby perpetuating the damaging cellular effects (Han et al., 2011).

3.2 Adaptive Immune Response

Growing evidence supports an association between neuropathologies of the CNS and immune changes in the periphery. Although the innate immune response in the CNS can influence the immune status of the organism as whole, we now know that immune activation in the periphery can also affect neuronal cell survival. Peripheral immune responses have been linked to many CNS disorders including PD, Alzheimer's disease (AD), multiple sclerosis (MS), amyotrophic lateral sclerosis (ALS), stroke, traumatic brain injury (Beschorner et al., 2002; Brochard et al., 2009;

Combrinck et al., 2002; Cunningham et al., 2005; Miklossy et al., 2006; Rentzos et al., 2012). In addition, aging has been reported to be a risk factor in a discordant link between the peripheral immune system and the CNS (Godbout et al., 2005). These studies all suggest that activated or primed microglia mount an exaggerated response to a secondary stimuli compared to resting microglia, thereby furthering contributing to the neuropathologies and their associated behavioural defects.

Under normal physiological conditions, activated T- and B-lymphocytes are not present in high numbers in the CNS. However, following infection or injury in the CNS and the subsequent induction of the innate immune response, the production of pro-inflammatory cytokines such as IL-1β and TNF-α by glial cells is increased. This increases the permeability of the BBB, leading to the upregulation in cellular adhesion molecules on microvascular endothelial cells. Activated T cells and B cells then migrate to the site of neuronal injury. In addition, there is an upregulation in chemokines within the brain leading to increased migration of peripheral leukocytes. It is widely considered that acute neuroinflammation is beneficial in the CNS after injury or infection by ensuring homeostasis.   However, chronic neuroinflammation is known to be damaging to the CNS and this can be attributed in part, to the generation of ROS **(Figure 1).**

4.   MECHANISMS OF OXIDATIVE STRESS AND NEUROINFLAMMATION IN PARKINSON'S DISEASE

Oxidative stress is a cytotoxic condition that occurs when there is an increased intracellular overproduction, or accumulation of ROS in conjunction with reduced antioxidant capacity within the cell. The most common species of ROS include molecules such as superoxide (O_2^-) and hydrogen peroxide (H_2O_2). Additionally, H_2O_2 is associated with the production of the reactive nitric oxide (NO) species, which can also react with O_2^- to produce peroxynitrite (PN) which is an extremely active oxidising agent (Chinta et al., 2011). In the CNS, microglia are a major source

of ROS through intracellular peroxidases, oxidative processes in mitochondria and NADPH oxidase activity at the cell-surface membrane (Block et al., 2007). ROS play a role in facilitating some normal cell functions such as proliferation and survival (Le Belle et al., 2011). Under healthy conditions, O_2^- and H_2O_2 are normal by-products of oxygen metabolism by the mitochondria. The generation of O_2^- from mitochondrial complexes I and III of the electron transport chain can be subsequently reduced to H_2O_2 that can then be decomposed by catalases and glutathione peroxidases. However, in both acute and chronic conditions of the brain where there are reduced levels of enzymatic antioxidants, the generation of oxidative stress occurs via the release of H_2O_2 into the cytosol. Furthermore, extracellular and environmental insults can stimulate the production of ROS and oxidative stress. At high levels of ROS production, proteins become inactivated or damaged which can modulate intracellular signalling pathways leading to cellular degeneration and death. In addition, ROS can initiate pro-inflammatory pathways, further perpetuating the deleterious environment for vulnerable neuronal populations.

Evidence suggests that both oxidative stress and neuroinflammation contribute to the neuronal degeneration observed in PD (Hirsch et al., 2012; Varcin et al., 2012). The DA neurons in the SN are highly susceptible to oxidative damage due to the high oxygen consumption of this brain region together with the low levels of antioxidant enzymes such as superoxide dismutase (SOD), glutathione (GSH) and catalase (Floyd, 1999). The generation of ROS can also be increased in the PD brain due to the synthesis and storage of dopamine. Dopamine (DA) is stable in synaptic vesicles inside the cell, however once DA exists outside the vesicle in a damaged neuron, it is easily metabolised by monoamine oxidase (MAO). Alternatively, DA can undergo auto-oxidation in the oxygen rich brain environment to produce cytotoxic ROS. In the oxidation of DA by MAO, H_2O_2 and dihydroxyphenylacetic acid (DOPAC) are generated. However, when dopamine is auto-oxidized it produces reactive quinones and O_2^-. The O_2^- is can be subsequently converted to H_2O_2 by SOD or react with nitric oxide (NO) radicals to generate peroxynitrite. It has been proposed by *in vitro*

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studies that L-Dopa can induce DA neuron cell death through the generation of ROS however this is still strongly debated (Coyle et al., 1993; Lipski et al., 2012).

Post mortem evidence has demonstrated a marked reduction in GSH levels, decreased mitochondrial complex I activity, increased SOD activity and elevated levels of free iron in the PD brain (Fahn et al., 1992). The high rates of catecholamine metabolism in the midbrain are associated with increased levels of neuromelanin in this brain region. When released from dying cells, neuromelanin is a potent activator of microglia, increasing the sensitivity of DA neurons to oxidative stress-mediated cell death (Halliday et al., 2005; Kastner et al., 1992; Zhang et al., 2011). Specifically, it has been proposed that the high levels of iron in the basal ganglia that can interact with both neuromelanin and DA may be a key factor in the initiation of the neuronal cell death (Gerlach et al., 1994). ROS can also initiate excitotoxicity through the over activation of N-methyl-D-aspartate (NMDA) receptors leading to an upregulation in NO (Barnham et al., 2004). Human post-mortem PD brains display increased oxidative damage to lipid, proteins and DNA (Mythri et al., 2011). These can further activate microglia through DAMP-associated pathways and indeed this has been shown with α-synuclein (Beraud et al., 2012). The SN has a higher density of microglia compared with other regions of the brain, therefore the detrimental effects of microglia activation can be even more pronounced in PD (Lawson et al., 1990). In addition, compared to cortical or hippocampal neurons, midbrain DA neurons are more susceptible to pro-inflammatory cytokines such as TNFα (McGuire et al., 2001). This increased sensitivity has been linked to elevated oxidative stress generated through the Fenton reaction.

5. EVIDENCE FOR NEUROINFLAMMATION IN PARKINSONS DISEASE

Evidence from both environmental and genetic models supports the involvement of oxidative stress in all pathological events associated with PD, including protein

misfolding and aggregation, UPS impairment, mitochondrial dysfunction and abnormal signaling transduction. Epidemiological, human post mortem, and animal studies have all implicated the innate neuroinflammatory response in PD and recent studies also suggest a role for peripheral immune cells in the disease pathogenesis.

5.1   Clinical Studies

McGeer et al., (1988) first provided evidence of neuroinflammation in human PD in a post mortem study over two decades ago with increased numbers of major histocompatibility complex (MHC) class II-positive (activated) microglia identified in the SN of patients. A later study also reported an elevation in the light chain of MHC class I, β2-microglobin in the striatum of PD patients (Mogi et al., 1995). The use of PET imaging with the ligand PK11195 has recently confirmed the presence of activated microglia in the human post-mortem PD brain (Gerhard et al., 2006). Subsequent clinical studies have provided further evidence for the involvement of a neuroinflammatory response in the pathology of PD **(Table 1)**. Studies have confirmed elevated levels of a host of pro-inflammatory cytokines, TNF-α, IL-1β, IL-6, IL-2, IL-4 and IL-6 in the post mortem brain and/or CSF (Hunot et al., 1999; Mogi et al., 1996; Mogi et al., 1994). Recently, a microarray study reported the increased expression of genes encoding both pro-inflammatory cytokines and subunits of the mitochondrial respiratory chain in the SN of PD brains compared to control brains (Duke et al., 2007). The adaptive arm of the immune response has also been implicated in PD, with studies showing CD4+ and CD8+ T cells invading the brain in post mortem human PD brains (Brochard et al., 2009). Additionally, increased levels of IFN-γ positive cells are observed in the SN of PD brains in the presence of lymphocytes. This suggests that the infiltration of immune cells across the blood brain barrier may also participate in the neuroinflammatory events (Hirsch et al., 2005).

5.2 Epidemiological

Epidemiological studies provide compelling evidence that inflammatory mechanisms contribute to the onset of PD. The incidence of sporadic PD in chronic users of non-steroidal anti-inflammatory drugs (NSAIDs), which scavenge oxygen free radicals and inhibit COX activity, was reported to be significantly lower (46%) than that of age matched non-users (Chen et al., 2003). This finding was similar to a follow up study for chronic users of the non-selective COX inhibitor ibuprofen. This reduced incidence has been linked to the inhibition of COX mediated DA oxidation and reduced production of microglial-derived toxins (Chen et al., 2005; Chen et al., 2003). However, other studies have reported contradictory findings of little or no effect of NSAIDs in decreasing the risk of PD (Hancock et al., 2007; Hernan et al., 2006). However, these studies do suggest that early intervention with NSAIDs or other anti-inflammatory therapies may be effective in delaying the onset of PD. Indeed, a recent study did report a correlation between elevated plasma IL-6 levels and an increased risk of PD (Chen et al., 2008).

Epidemiological studies have also suggested a link between brain injuries and the development of PD later on in life. Triggers known to promote a neuroinflammatory response including traumatic brain injury, exposures to viruses and infectious agents have all been proposed as increased risk factors for developing PD (reviewed in (Tansey et al., 2010)). In addition, neurotoxins such as 1-methyl-4-phenyl-1, 2, 3, 6-tetrahydropyridine (MPTP) and the pesticides rotenone and paraquat are commonly used to recapitulate PD in animal models and display features of neuroinflammation **(Table 2)**.

5.3 Toxin based models of PD

5.3.1 MPTP

MPTP is commonly used to induce PD in both rodents and primates. It is a lipophilic protoxin that following systemic injection, rapidly crosses the BBB where it is converted by MAO-B (by glial and serotoneric neurons) to 1-methyl-4-phenylpyridinium (MPP+) (Riachi et al., 1989). MPP+ is then released into the

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extracellular space where it is taken up via the dopamine transporter DAT into the DA neurons, primarily accumulating in the mitochondria. Here, it interferes with complex I of the mitochondrial respiratory chain, leading to reduced ATP production and increased ROS, and the subsequent activation of cell death pathways (Williams et al., 2005). Many species, including rats are insensitive to MPTP and this has been attributed to the rapid clearance of MPP+ (Johannessen et al., 1985). However, specific strains of mice, including the commonly used C57Bl strain are sensitive to MPTP-induced toxicity, displaying many features of the human disease. There is bilateral degeneration of the nigro-striatal tract with reductions in striatal dopamine and tyrosine hydroxylase (TH). Levels of extracellular glutamate and glutathione are increased and decreased, respectively in the SN of MPTP-treated mice (Ferraro et al., 1986; Meredith et al., 2009). MPTP also induces a glial response in mice that has been shown to peak prior to the loss of the DA neurons (Czlonkowska et al., 1996). This includes increased levels of inflammatory cytokines (including IFNγ) and microglial activation (Smeyne et al., 2005). There is also the involvement of the adaptive immune response with increased lymphocyte infiltrates identified (Kurkowska Jastrzebska et al., 1999), A number of studies have further investigated the role of these pro-inflammatory cytokines in mediating the neuronal cell death in MPTP models. Mice lacking IFNγ or the receptors for TNFα display attenuated nigro-striatal degeneration induced by MPTP (Mount et al., 2007; Sriram et al., 2002). A later study used IFNγ and TNFα knockout mice to identify a synergistic role between these cytokines in mediating microglia and astrocytic activation and thus neuronal cell death in an MPTP model (Barcia et al., 2011). Increased expression of IFNγ and TNFα was also identified in microglia and astrocytes of the SN of MPTP-treated primates (Barcia et al., 2011). Significantly, the levels of microglia IFNγ correlated with the motor impairment and DA degeneration in these primates. Increased levels of iNOS have also been reported in mice injected with MPTP, while mice lacking the gene for iNOS display protection against MPTP-induced DA degeneration (Liberatore et al., 1999).

The ability of anti-inflammatory agents to block the cell death in the MPTP model has been tested with variable success. The microglial inhibitor, minocycline has been shown to confer protection when given multiple times during the MPTP injection procedure (Du et al., 2001), however when administration was extended over a couple of days, this protective effect was not seen (Yang et al., 2003). Possible explanations for these discrepancies may be the potential ability of minocycline to block MPTP metabolism (thereby providing protection) and the selectivity of minocycline for microglia that would not target the pro-inflammatory astrocytic response.

5.3.2   6-OHDA

A neurotoxic analogue of dopamine, 6-hydroxydopamine (6-OHDA) has also been used to induce nigro-striatal degeneration in rodents. Unlike MPTP, it does not efficiently cross the BBB and therefore it must be injected directly into the nigro-striatal tract where it is taken up into the DA neurons via DAT. The ability of 6-OHDA to induce DA degeneration has been linked to oxidative stress mechanisms and mitochondrial respiration dysfunction (Barnum et al., 2010). 6-OHDA is readily oxidized to form ROS such as H_2O_2 (Mazzio et al., 2004) and has also been shown to reduce striatal levels of GSH and SOD (Kunikowska et al., 2001; Perumal et al., 1992), increase iron levels in the SN (Oestreicher et al., 1994) and to directly interact and inhibit the mitochondrial respiratory chain (Glinka et al., 1997).

The 6-OHDA model displays many of the pathological and biochemical features of PD. Reduced levels of striatal dopamine and TH accompany the decreased numbers of TH-positive neurons in the SNpc. In addition, 6-OHDA lesioned mice also display features of neuroinflammation with PET studies with the ligand PK11195 confirming increased numbers of activated microglia in the striatum (Cicchetti et al., 2002). This microglial activation can be blocked by minocycline with a subsequent delay in DA cell loss (He et al., 2001). A similar protective effect in attenuating microglial activation was reported with the COX-2 inhibitor, celecoxib (Sanchez-Pernaute et al., 2004). 6-OHDA has also been linked to elevated levels of pro-

inflammatory mediators such as TNFα (Mogi et al., 2000). Indeed, a study blocking the soluble form of the TNFα receptor reported attenuation in the death of DA neurons in 6-OHDA lesions rats (McCoy et al., 2006).

5.3.3 *Rotenone and paraquat*

A number of environmental toxins including the herbicide paraquat and the pesticide rotenone have been linked to an increased risk of PD. Epidemiological studies have reported a higher incidence of PD in farmers exposed to these toxins (Baldereschi et al., 2008; Hancock et al., 2008; Kamel et al., 2007). Rotenone is highly lipophilic, easily crossing the BBB where it diffuses into neurons to inhibit complex I of the mitochondrial respiratory chain. Administration of rotenone results in the neurodegeneration of the nigrostriatal dopaminergic system however its selectivity has been questioned (Betarbet et al., 2000; Cicchetti et al., 2009). The toxicity of rotenone has been linked to increased ROS production with oxidative damage reported in the midbrain and striatum of rotenone treated rats (Sherer et al., 2003). In addition, consistent with the human disease, rotenone treated rats display increased microglial activation (OX-42 immunoreactivity) in the SN and striatum (Sherer et al., 2003b). Significantly, this occurred prior to DA cell loss in these rats. *In vitro* models of rotenone-induced toxicity have also implicated an important role for microglia in mediating this response. Mount et al., (2007) used a microglia/midbrain neuron culture system to implicate IFN-γ in mediating the microglial response detrimental to rotenone-induced neuronal cell death *in vitro*. Evidence also suggests microglia play a critical role in mediating rotenone-induced neuronal degeneration through NADPH oxidase-generated superoxide (Gao et al., 2003). Importantly, the selective inhibition of microglia by minocycline or iptakalim has been shown to confer some protection against neuronal cell death following exposure to rotenone (Casarejos et al., 2006; Zhou et al., 2007).

Paraquat is a non-selective bipyridyl contact herbicide and a charged molecule that does not cross the BBB. Unlike, MPP+ that utlilises DAT, paraquat requires a neutral

amino acid transporter (such as the system L carrier, LAT-1) to transport it into neurons (Shimizu et al., 2001). In the cytosol, it generates oxidative stress (superoxide formation) and also impairs the recycling of oxidized glutathione. Paraquat is highly selective for DA neurons and indeed the intracerebral injection of paraquat has been shown to induce features of PD including a loss of DA neurons and depletion in levels of striatal dopamine (Liou et al., 1996). Evidence of microglial activation has been reported in mice treated with paraquat (Purisai *et al.*, 2007). Recently, it has been reported that IFNγ knockout mice display reduced microglial activation, decreased pro-inflammatory enzymes (iNOS and COX-2) and cytokines (TNFα, IL1β) and increased trophic factors compared to wildtype mice. This correlated with a reduction in paraquat-induced cell death in the IFNγ-/- mice (Mangano et al., 2012). Studies have increased the toxicity of paraquat by co-administering the fungicide, Maneb (manganese ethylene-bis-dithiocarbamate), an inhibitor of complex III of the mitochondrial respiratory chain (Thiruchelvam et al., 2000). Significant microglia activation, increased iNOS and lipid peroxidation has also been reported in this model (Cicchetti et al., 2005; Gupta et al., 2010).

5.4   Genetic models

5.4.1   SCNA (PARK1/PARK4)

The SCNA gene encodes α-synuclein (α-syn), a 140 amino acid synaptic vesicle protein located in the cytoplasm or within lipid membranes. It has been implicated in neurotransmitter release, vesicle turnover, synaptic plasticity, and endoplasmic reticulum trafficking (reviewed in (Gao et al., 2011)). Dominantly inherited mutations (A30P, A53T and E46K) or gene multiplications in the SCNA gene that increase α-syn expression have been linked to familial PD. α-syn is a natively unfolded soluble protein, however under certain conditions, it can aggregate to form oligomers or protofibrils and eventually insoluble polymers or fibrils (Conway et al., 2000). Both forms have been shown to be neurotoxic, through an inhibition of complex I activity and elevated oxidative stress (Sherer et al., 2003). However, it

has been suggested that the sequestration of the intermediate forms into the mature fibrils may be a protective mechanism. Indeed, misfolded α-syn is a major component of Lewy bodies in both familial and sporadic forms of PD (Spillantini et al., 1997) and missense mutations have been linked to an increased propensity of α-syn to misfold (Conway et al., 2000). The stabilisation of α-syn protofibrils by free radicals has been reported while their aggregation can in turn induce oxidative stress (Junn et al., 2002). Recently, it has been suggested that the neuronal cell death and subsequent release of these protein aggregates can activate nearby microglia (Roodveldt et al., 2008). Aggregated and nitrated or oxidised α-syn has been reported to activate and be phagocytosed by microglia *in vitro* (Roodveldt et al., 2008; Zhang et al., 2005). This subsequently leads to an activation of NADPH oxidase and increased production of ROS, specifically superoxide (Zhang et al., 2005). In addition, evidence suggests that nitrated α-syn cannot only activate inflammatory and redox responses in microglia (Reynolds et al., 2008), it can also activate the adaptive immune response with increased leukocyte infiltration (Benner et al., 2008).

A number of SCNA mouse models (knockouts, overexpressers and transgenics) have been generated carrying different mutations (reviewed in (Antony et al., 2011; Chesselet, 2008)). Although there is a lack of consistent DA cell loss in any of these models, they do display various CNS abnormalities, including α-syn aggregation, gliosis, mitochondrial abnormalities and slight functional abnormalities in the nigro-striatal system (reviewed in (Dawson et al., 2010)). Increased α-syn aggregation and oxidative damage has been reported in mice that overexpress mutant or wildtype α-syn, with oxidised and nitrated α-syn identified in their brains (Giasson et al., 2002). Proteomic analysis of brains from mice overexpressing the human A30P mutation identified increased levels of oxidised metabolic proteins (Poon et al., 2005), while the mutant A53T mice display mitochondrial damage and degeneration (Martin et al., 2006). Recently, a study of mice over-expressing wild-type α-syn under the Thy1-promoter (Thy1-αSyn mice), reported increased microglia activation and elevated levels of TNFα in the striatum (1 month of age) and SN (5–6 months) (Watson et al., 2012). TLR1, TLR4 and TLR8 expression was also

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increased at 5–6 months in the SN suggesting a pro-inflammatory response in these mice. In support of a further link between α-syn and a neuroinflammatory response, IP injection of LPS into human A53T mice led to a persistent neuroinflammatory response compared to wildtype mice (Gao et al., 2011). This was accompanied by the accumulation of nitrated α-syn and Lewy body-like inclusions and the degeneration of the nigrostriatal system. Significantly, inhibitors of iNOS and NADPH oxidase were able to reduce both microglia activation and the nigral degeneration.

5.4.2 LRRK2 (PARK8)

Leucine-rich-repeat-kinase 2 (LRRK2) contains 51 exons encoding a 2527 amino acid protein containing a functional kinase and GTPase domain, and the Leucine-rich repeat (LRR) and WD40 protein-interaction domains (Paisan-Ruiz et al., 2004; Zimprich et al., 2004). Multiple functions for LRRK2 have therefore been suggested including protein scaffolding, substrate binding and protein phosphorylation (reviewed in (Drolet et al., 2011)). It is expressed throughout various brain regions, including the SN, basal ganglia, cortex, hippocampus and cerebellum (Biskup et al., 2006; Healy et al., 2008). Mutations in *LRRK2* are the most common cause of familial PD and have been linked to both the autosomal dominant and sporadic forms of the disease (Correia Guedes et al., 2010). The most prevalent modification of LRRK2 is the amino acid substitution G2019S in the kinase domain, generating a pathogenic gain-of-function. Overexpression of G2019S has been linked to enhanced LRRK2 autophosphorylation and kinase activity (Greggio et al., 2006; Li et al., 2010). In neurons, LRRK2 has been described as a potent regulator of the cytoskeleton, with knockdown of LRRK2 protein enhancing neurite outgrowth and mutant over expression inhibiting outgrowth (MacLeod et al., 2006). This has been attributed to the dis-organisation of the microtubule and actin cytoskeletons, arising from substrate phosphorylation (Gillardon, 2009; Parisiadou et al., 2009). Furthermore, there is significant *in vitro* evidence that LRRK2 over expression causes neurotoxicity (Li et al., 2010; Smith et al., 2006; West et al., 2007).

Several *in vitro* studies have suggested a link between LRRK2 and oxidative stress. It was first proposed by West *et al.*, (2007) that the enhanced kinase activity of LRRK2 mutants increased neurons susceptibility to H_2O_2 *in vitro*. Wildtype LRRK2 has been shown to protect against H_2O_2 induced-cell death in HEK293 and SY5Y cells through an ERK-dependent pathway (Liou et al., 2008). In contrast, overexpression of the Y1699C or G2019S mutants failed to confer protection. Similarly, G2019S overexpressing dopaminergic SN4741 cells displayed an increased susceptibility to H_2O_2 compared to wildtype LRRK2 overexpressing cells and this was linked to the enhanced kinase activity of the mutant (Heo et al., 2010). More recently the connection between LRRK2 and oxidative stress was established through defects in the morphology and dynamics of the mitochondria in cortical neurons (Niu et al., 2012). The disruption in mitochondrial fission/fusion has been linked to increased ROS production in the mutant cells. It has also been reported that LRRK2 can interact with peroxiredoxin-3 (PRDX3), a mitochondrial anti-oxidant. Significantly, mutations in LRRK2 resulted in the increased phosphorylation of PRDX3 and subsequent mitochondrial dysfunction (Angeles et al., 2011). Indeed, decreased mitochondrial membrane potential and total intracellular ATP levels have been reported in skin biopsies from G2019S mutation carriers suggesting impaired mitochondrial function (Mortiboys et al., 2010). A recent study also reported that induced pluripotent stem cells (iPSC) that carry the G2019S mutation are more sensitive to caspase-3 activation and cell death caused by exposure to H_2O_2, proteasomal inhibition (MG-132) and 6-OHDA than control DA neurons (Nguyen et al., 2011).

Recent *in vitro* studies have also implicated LRRK2 in the innate immune response. A potential role for LRRK2 in the immune response was first proposed when it was found to be expressed in B-lymphocytes, dendritic cells and macrophages (Gardet et al., 2010). In addition, a genome wide study identified *LRRK2* as a susceptibility locus for Crohn's disease (Danoy *et al.*, 2010). Recently, *LRRK2* was shown to be an IFN-γ target gene in a macrophage cell line (Gardet et al., 2010). However, of greater

interest was the recent report describing LRRK2 expression in isolated mouse microglial cells (Gillardon et al., 2012). LRRK2 protein levels were upregulated by LPS with LRRK2 (1441G) transgenic microglia secreting higher levels of TNF-α, IL-1β and IL-6 and lower amounts of anti-inflammatory IL-10, compared to wildtype microglia. The potential neurotoxic effects of this pro-inflammatory response were confirmed when the culture medium of the LPS stimulated cells was added to cultured cortical neurons (Gillardon et al., 2012). Other studies have also confirmed a link between the G2109S LRRK2 mutation and an elevated LPS induced-inflammatory response that includes TNF-α, IL-1β, IL-6, NF-Kβ and nitric oxide synthase (iNOS) secretion (Kim et al., 2012; Moehle et al., 2012). Furthermore, knockdown of LRRK2 in microglia reduced LPS-induced TNF-α and iNOS production, and also decreased activation of the p38 and NFκB pathways. Recently, the phosphorylation of LRRK2 was shown to be through a TLR-mediated pathway involving Myd88 further confirming a role for LRRK2 in the innate immune response (Dzamko et al., 2012).

LRRK2 knockout mice have been generated however the majority of models fail to display any overt neuronal cell loss (reviewed in (Yue et al., 2011)). Furthermore, the recent report that mice lacking LRRK2 demonstrate a similar susceptibility to MPTP as wildtype mice suggests that a loss of LRRK2 does not cause PD (Andres-Mateos et al., 2009). A number of groups have reported BAC transgenic mice expressing the common G2019S and R1441G LRRK2 mutations or their murine forms and these do demonstrate subtle phenotypes. Recently, Li *et al*. (2010) compared the phenotypes of mice overexpressing wildtype LRRK2 (WT-LRRK2) or the murine form of the LRRK2-G2019S mutation. WT-LRRK2 mice displayed elevated striatal dopamine release with unaltered DA uptake or tissue content and were hyperactive with enhanced performance in motor function tests (Li et al., 2010). In contrast, an age-dependent decrease in striatal DA content as well as decreased striatal DA release and uptake was identified in the G2019S-LRRK2 mice. However, these mice did not show any motor function abnormalities up to 12

months of age. This suggests a critical role for LRRK2 in mediating striatal dopamine transmission and therefore motor function control. Transgenic mice expressing human WT-LRRK2 or the PD mutations G2019S-LRRK2 or R1441C-LRRK2 have also been generated. Melrose et al., (2010) reported a reduction in extracellular dopamine in both WT-LRRK2 and G2019S-LRRK2 mice with abnormal localisation and phosphorylation of the microtubule binding protein tau, however no loss in DA neurons was evident. In contrast, progressive nigro-striatal degeneration has been reported in human G2109S-LRRK2 mice (Ramonet et al., 2011). Reduced levels of cortical catecholamines and a progressive impairment of locomotor activity were also reported in aged R1441C-LRRK2 transgenic mice. This supported an earlier study with human R1441G-LRRK2 transgenic mice, which display signs of axonal dystrophy and degeneration and also develop an age-dependent and levodopa-responsive movement abnormality (Li et al., 2009). Importantly, *C. elegans* models overexpressing the human G2019S or R1441C mutations also display age-related degeneration, reduced dopamine levels and behavioural defects and an enhanced vulnerability to rotenone (Saha et al., 2009; Yao et al., 2010). Similarly, *Drosophila* overexpressing LRRK2 are more sensitive to rotenone (Venderova et al., 2009)

Although the DA cell loss seen in the mice models is not dramatic, they do support a role of LRRK2 in a number of pathogenic pathways implicated in PD, including oxidative stress and neuroinflammation. Mice overexpressing G2019S-LRRK2 display an age-related increase in mitochondrial autophagy and damage in the cerebral cortex (Ramonet et al., 2011). This supports *in vitro* studies implicating LRRK2 mutations in abnormal autophagy and subsequent neurite shortening (Plowey et al., 2008). Indeed, Ramonet *et al.,* (2011) did report reduced neurite complexity in DA neurons cultured from the G2019S-LRRK2 mice. Furthermore, mitochondrial abnormalities (as well as DA degeneration and UPS and microtubule dysfunction) in the A53T α-syn mouse are exacerbated by the overexpression of G2019S-LRRK2 (Lin et al., 2009). In addition, the A53T/G2019S-LRRK2 mice display increased astrocytosis and microgliosis. Other LRRK2 models have also

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provided supporting evidence for the pathogenic role of LRRK2 mutations in PD. *Drosophila* lacking the kinase domain of LRRK2 develop normally, however they are more sensitive to H_2O_2 induced stress (Wang et al., 2008). A later study with G2109S overexpressing Drosophila reported increased cell toxicity induced by both sub-toxic and toxic H_2O_2 doses compared to their wild type LRRK2 counterparts and this was attributed to the enhanced kinase activity of the mutants (Yang et al., 2012). Significantly, this effect was inhibited by curcumin, an antioxidant compound shown to protect the brain from lipid peroxidation and to scavenge NO-based radicals (Shimura et al., 2012; Sreejayan et al., 1997). These studies highlight the important balance between the normal physiological role of LRRK2 and the potential pathogenic mechanisms underlying the enhanced kinase activity of the G2019S mutation.

5.4.3   Parkin (PARK2)
Parkin encodes a 465aa protein comprised of a N-terminal ubiquitin-like domain, an IBR (in-between-ring) domain and two RING-finger domains. Loss of function mutations in *parkin* are the most common cause of early onset autosomal recessive PD (AR-PD) (Lucking et al., 2000). In addition, heterozygous *parkin* mutation carriers can also display parkinsonian features or can present with the late-onset form of the disease (Dawson et al., 2010b). Parkin functions as an E3-ubiqutin ligase conjugating ubiquitin to misfolded or toxic proteins for degradation by the 26S proteasome (Shimura et al., 2000). Parkin can ubiquitinate proteins at both K48- and K63-lysine linkages and it is now known that it plays a critical role in mitophagy; the removal of damaged or dysfunctional mitochondria by autophagy (Narendra et al., 2008). Many of the PD-causing mutations in *parkin* abolish or reduce its E3 ubiquitin ligase activity or alternatively affect substrate specificity (Dawson and Dawson, 2010b). To date, greater than 15 substrates of parkin have been reported, implicating the protein in numerous cellular functions/pathways and these have been extensively reviewed elsewhere. *In vitro* studies first provided the link between parkin and oxidative stress over a decade ago. Overexpression

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of mutant parkin (Del 3-5, T240R, and Q311X) in neuroblastoma cells was reported to increase levels of protein carbonyls, lipid peroxidation and also nitrated proteins (Hyun et al., 2002). In a later study by the same group, overexpression of wild-type parkin was shown to delay cell death induced by serum withdrawal, H_2O_2, MPP(+) or 4-hydroxy-2-trans-nonenal (HNE) and this corresponded with a decrease in protein oxidation (Hyun et al., 2005). In contrast, overexpression of PD associated parkin mutants accelerated cell death and led to increased protein oxidation, lipid peroxidation, and 3-nitrotyrosine levels and decreased GSH levels.

An important role for parkin in glial cells has also been proposed by a number of *in vitro* studies. Midbrain neurons cultured from parkin knockout mice are more sensitive to rotenone (Casarejos et al., 2006). Significantly, the same study showed that the addition of knockout microglia to wildtype neurons increased their sensitivity to rotenone. A later study reported that aged parkin knockout glia behave differently in culture with less astrocytes, more microglia, reduced proliferation, and increased pro-apoptotic protein expression (Solano et al., 2008). This would promote the release of pro-inflammatory cytokines and *in vivo*, promote neuronal cell death. In support of this, macrophages isolated from parkin knockout mice display increases in TNFα, IL1-β and iNOS mRNA expression (Tran et al., 2011). In addition, a downregulation in parkin expression in response to LPS and TNFα was reported by this study in macrophages, microglia and neurons.

Parkin knockout mice do not display any overt dopaminergic cell loss however behavioural and motor function abnormalities together with alterations in nigrostriatal dopamine have been reported in the various models. Increased protein oxidation and lipid peroxidation has been reported in the brains of parkin knockout mice at 18-20 months of age (Palacino et al., 2004). In addition, a reduction in proteins involved in mitochondrial respiration was identified in the parkin knockout brains and this correlated with reduced electron transport chain capacity. Rodriguez-Navarro et al., (2007) also analysed aged parkin knockout mice and

found they are unable to compensate for the increasing oxidative stress. For example, while young mice have increased total GSH levels in their brains, levels are decreased in aged parkin knockout mice. In addition, they display increased astrogliosis in the striatum and increased microglial activation in the midbrain. Recently the overexpression of human mutant parkin has been reported to show a convincing phenotype (Lu et al., 2009). This study used a BAC transgenic mouse model expressing C-terminal truncated human mutant parkin (Parkin-Q311X) driven by a dopamine transporter promoter. These mice not only developed age-dependent DA neuron degeneration in the SN reduced striatal dopamine, they also exhibited progressive hypokinetic motor deficits. Furthermore, the Parkin-Q311X mice displayed evidence of elevated oxidative damage in the SN.

These genetic models have also been used in concert with the toxin-based models mentioned previously to further understand characterise parkin's involvement in the oxidative stress and neuroinflammatory response in PD. Indeed, parkin knockout mice are more susceptible to rotenone induced DA degeneration, however somewhat surprisingly they display a similar response to 6-OHDA as their wildtype counterparts (Perez et al., 2005). Parkin knockout mice do display increased nigral degeneration following systemic LPS administration, however the mechanisms underlying this susceptibility are unknown as oxidative stress and pro-inflammatory markers were unchanged (Frank-Cannon et al., 2008). Evidence suggests that nitric oxide exacerbates the s-nitrosylation of parkin in the MPTP model thereby inhibiting its E3 ubiquitin ligase activity (Chung et al., 2004). Although parkin knockout mice are not more susceptible to MPTP (Zhu et al., 2007), it was recently reported that the overexpression of wildtype parkin protects mice from DA degeneration. This was attributed in part to an improvement in mitochondrial impairment induced by this toxin (Bian et al., 2012).

5.4.4 PINK1(PARK6)

The *PTEN-induced putative kinase-1* (PINK1) gene is comprised of 8 exons and encodes a 581 amino acid serine/threonine protein kinase with significant

homology to the calcium/calmodulin-dependent protein kinase (Valente et al., 2004). It is mainly localized to the mitochondria and therefore the majority of studies have focused on its role in mitochondrial functions, specifically calcium dynamics, trafficking, ROS generation, respiration and the opening of the mitochondrial permeability transition pore. Mutations in PINK1 are causative for early onset AR-PD but an involvement for heterozygous PINK1 mutations in late-onset PD has also been suggested (reviewed in (Bonifati, 2012)). The loss-of-function mutations in PINK1 have been shown to influence its stability, localization and kinase activity (Beilina et al., 2005; Valente et al., 2004). *In vitro* studies have confirmed a critical role for PINK1 in protecting against oxidative stress-induced cell toxicity. Overexpression of wildtype PINK1 has been shown to protect SH-SY5Y cells from both basal and staurosporine-induced cell death, with reduced cytochome c release and caspase-3 (and -7 and -9) activation (Petit et al., 2005). In contrast, this protection was not seen in cells overexpressing PD-associated mutations in PINK1. Pink1 knockout mice are more susceptible to DA degeneration and striatal DA depletion induced by MPTP, but significantly, this can be rescued by the viral mediated expression of parkin or DJ-1 (Haque et al., 2012).

5.4.5 DJ-1(PARK7)

The DJ-1 gene contains 8 exons and encodes a molecular chaperone that is localised to the cytosol and the mitochondria (reviewed in (Cookson et al., 2010)). DJ-1 has been shown to regulate redox-dependent kinase signaling pathways and antioxidant expression (Kahle et al., 2009). Mutations in DJ-1 are rare and have been linked to less that 1% of EO-PD cases (Clark et al., 2004). However, patients with mutations in DJ-1 do share phenotypic similarities to other familial PD patients and evidence suggests that the protein functionally interacts with parkin and PINK1. Studies with these genetic mouse models support the notion that common pathways are affected in the familial (and sporadic) forms of the disease with oxidative stress **(Table 3)** and neuroinflammation **(Table 4)** playing central roles. Indeed, recent evidence has suggested that DJ-1 acts in parallel to the parkin-PINK1 pathways to control mitochondrial polarization and morphology (Irrcher et al., 2010; Thomas et al.,

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2011). Like parkin and PINK1, a role for DJ-1 in mitophagy has also been proposed with cells deficient in DJ-1 displaying increased oxidative stress, mitochondrial fragmentation and dysfunction and elevated levels of autophagy (Thomas *et al.*, 2011). Significantly, overexpression of parkin and PINK1 was able to rescue the mitochondrial phenotype.

Recently, Rousseaux et al., (2012) identified an early PD phenotype (8 weeks of age) in DJ-1 knockout mice on a pure C57Bl/6J background that progressed with age and was of incomplete penetrance.   These mice displayed unilateral nigral degeneration that was accompanied by dysmorphic neurites and microgliosis. The cause of this phenotype was unknown as the majority of studies have failed to report any DA degeneration or behavioural abnormalities in DJ-1 KO mice (Andres-Mateos et al., 2007; Manning-Bog et al., 2007). However, mitochondria from aged DJ-1 knockout mice do display a 2-fold higher level of H_2O_2 and a compensatory up-regulation in mitochondrial manganese SOD and GPx1 expression. In addition, mice deficient in DJ-1 are more susceptible to MPTP induced striatal dopamine depletion (Manning-Bog et al., 2007). DJ-1 has also implicated in the glial response in PD. Indeed, its expression is upregulated in reactive glial astrocytes in PD patients (Bandopadhyay et al., 2004). *In vitro,* LPS treatment of astrocyte-rich cultures from DJ-1 knockout mice leads to increase NO production and an upregulation in the pro-inflammatory mediators, COX-2 and IL-6 (Waak et al., 2009). In addition, the co-culture of DJ-1 knockout (but not wildtype) astrocytes enhanced LPS-induced cell toxicity of both wildtype and knockout cortical neurons.   Recently, apoptosis-signal regulating kinase 1 (ASK1) has been proposed as a critical link between oxidative stress and neuroinflammation. ASK1 is activated by oxidative stress, has been shown to be upregulated in the SN of PD brains and in the MPTP mouse model and significantly it is inhibited by DJ-1 (Lee et al., 2012). Importantly, reduced MPTP-induced COX-2 expression was recently reported in MPTP-treated ASK1 knockout mice (Lee et al., 2012). Crucially, COX-2 has been linked to microglial activation in this model of PD. In addition, ASK1 is necessary for the TLR4 innate immune response.   Not

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surprisingly, AKS1 knockout mice display reduced microglia and astrocyte activation and DA degeneration induced by MPTP.

Alternative models have also provided some insight into the pathways affected by these causative mutations in *parkin, PINK1 and DJ-1*. *Drosophila* parkin null mutants display partial lethality, decreased adult lifespan, apoptotic muscle degeneration and male sterility. This has been attributed to mitochondrial defects, oxidative stress and an early activation of the innate immune response (Greene et al., 2003). Aged parkin knockout flies show dopamine-responsive locomotion defects and degeneration of a subset of DA neurons (Whitworth et al., 2005). Significantly, the DA cell loss could be rescued by overexpression of the glutathione S-transferase S1 (*GstS1*) gene, further implicating oxidative stress in *parkin*-linked PD. Similar to the mouse model, *Drosophila* that overexpress mutant human parkin, display progressive DA cell degeneration (Sang et al., 2007). PINK1-null *Drosophila* display a similar phenotype to Parkin-null flies including male sterility behavioural abnormalities, mitochondrial defects and apoptotic muscle degeneration and increased sensitivity to oxidative stress (Clark et al., 2006). Significantly, overexpression of parkin rescued the muscle degeneration and mitochondrial abnormalities but not visa versa, suggesting the two proteins function in the same pathway with pink1 upstream of parkin (Park et al., 2006). Although knocking out of the *Drosophila* orthologs of DJ-1, DJ-1a and DJ-1b has no effect on DA neurons, the mutant *Drosophila* do display an increased sensitivity to paraquat and rotenone (Meulener et al., 2005). Furthermore, knocking out the DJ-1 ortholgue in *C. elegans* has suggested a key role for the protein in regulating the innate immune response through the p38 MAPK pathway (Cornejo Castro et al., 2010).

6. CONCLUSION

It is becoming increasingly evident that neuroinflammation and oxidative stress play a critical role in the aetiology of PD. In the title we have termed these two processes co-conspirators in the progression of PD as the molecular and

biochemical pathways that regulate both neuroinflammation and oxidative stress share common linkages and influence each other greatly. One of the functions of proinflammatory cytokines is to produce oxidative stress just as this resultant oxidative stress can subsequently shape the profile of cytokine release. The two processes are intertwined and cannot be easily separated and dealt with in isolation. With this in mind it raises the question of whether these processes are amenable to therapeutic intervention. Clearly with the current diagnosis of PD only occurring after vast DA cell death, targeting the neuroinflammatory component of the disease will not lead to the positive therapeutic outcomes required to combat the neural cell death that is manifest. However, with an earlier diagnosis of PD, limiting the early pro-inflammatory response will be beneficial in reducing further microglial activation and elevated oxidative stress that is key to slowing the cell death of susceptible neuronal populations.

Sampled region	Inflammatory cytokine/cell type	Activated microglia markers	Markers of ox. stress	Neurotrophins
Whole brain				
CSF	TNFα ↑ (Mogi et al., 1994) IL-1β ↑ (Blum-Degen et al., 1995; Mogi et al., 1996) IL-6 ↑ (Blum-Degen et al., 1995; Mogi et al., 1996) IL-2 = (Blum-Degen et al., 1995), ↑ (Mogi et al., 1996) IL-4 ↑ (Mogi et al., 1996) TGFa ↑ (Mogi et al., 1996)			
Serum	TNFa ↑ (Dobbs et al., 1999) IL-6 ↑ (Dobbs et al., 1999)			
SN	TNFα/microglia ↑ (Imamura et al., 2003) TNFα/glial cells ↑ (Boka et al., 1994) IL-6/microglia ↑ (Imamura et al., 2003) IFNγ ↑ (Mogi et al., 2007) NFκB ↑ (Mogi et al., 2007) Anti-inflammatory COX1, 2 ↑ (Knott et al., 2000)	HLA-DR (McGeer et al., 1988) CR3/43 and EBM11 (Banati et al., 1998) ICAM-1, LFA-1, MHC class II (Imamura et al., 2003)	NOS ↑ (Knott et al., 2000)	
Striatum	TNFα ↑ (Mogi et al., 1994), IL-1β ↑ (Mogi et al., 1994) IL-6 ↑ (Mogi et al., 1994) TGFα ↑ (Mogi et al., 1994)			GDNF ↑ (Mogi et al., 1994) = (Hunot et al., 1996) EGF ↑ (Mogi et al., 1994)
Hippocampus		MHC class II (Imamura et al., 2003)		
Caudate-putamen	IL-2 ↑ (Mogi et al., 1996) IFNγ ↑ (Mogi et al., 2007) NFκB ↑ (Mogi et al., 2007)	MHC class II (Imamura et al., 2003)		bFGF = (Mogi et al., 1996)
Cortexes	IL-1β = (Mogi et al., 1994) IL-2 = (Mogi et al., 1996) IL-6 = (Mogi et al., 1994) TGFα = (Mogi et al., 1994) IFNγ = (Mogi et al., 2007)	MHC class II (Imamura et al., 2003)		EGF = (Mogi et al., 1994)
Cerebellum	IFNγ = (Mogi et al., 2007)			
Mesencephalon	NFκB ↑ (Hunot et al., 1997)		↑ iNOS (Hunot et al., 1996)	GDNF = (Hunot et al., 1996)

Table 1: Evidence for neuroinflammation in human PD.

Alterations in inflammatory markers have been reported in human PD brain regions compared to control patients. ↑ represents an increase in expression and = represents no change in expression.

Mouse model of PD	Reactive microglia	MHC-class II	Pro-inflammatory cytokines	Chemokines	Inflitrating T-cells
MPTP acute/ subacute	(Barcia et al., 2011; Carta et al., 2009; Kohutnicka et al., 1998)↑	(Kurkowska-Jastrzebska et al., 1999; Yasuda et al., 2007) =	(Carta et al., 2011; Ciesielska et al., 2003; Kohutnicka et al., 1998; Lofrumento et al., 2011; Luchtman et al., 2012; Luchtman et al., 2009; Pattarini et al., 2007)↑	(Brochard et al., 2009; Kalkonde et al., 2007; Pattarini et al., 2007)↑	(Brochard et al., 2009; Kurkowska-Jastrzebska et al., 1999)↑
MPTP chronic	(Schintu et al., 2009)↑				
6-OHDA	(Pott Godoy et al., 2008)↑	(Pott Godoy et al., 2008)↑	(Arai et al., 2004; Pott Godoy et al., 2008)↑		
LPS	(Arai et al., 2004; Herrera et al., 2000)↑ (Iravani et al., 2005; Iravani et al., 2012)↑	(Pott Godoy et al., 2008)↑	(Pott Godoy et al., 2008)↑		
6OHDA+LPS	(Pott Godoy et al., 2008)↑	(Pott Godoy et al., 2008)↑	(Pott Godoy et al., 2008)↑		

Table 2: Evidence of a pro-inflammatory response in toxin-based mouse models of PD.

Alterations in inflammatory markers have been reported in mouse models of PD where ↑ represents an increase in expression and = represents no change in expression when compared to the wild-type control.

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Model	Age (months)	Marker of ox. stress	Marker of inflam.	Sampled region	Outcome vs control (neurodegeneration sensitivity)	Reference
DJ-1 KO	5, 11	Protein oxidation		Entire brain	←→	(Chen et al., 2005)
DJ-1 KO	2-3, 18-24	Protein oxidation Mitochondrial ROS Mitochondrial aconitase activity		Entire brain	←→ ↑ ↓ 2-3mo. ←→ 18-24mo.	(Andres-Mateos et al., 2007)
DJ-1 KO	24-27	DNA/RNA oxidation Nitrotyrosine 4-HNE		SN	←→ ←→ ←→	(Yamaguchi et al., 2007)
DJ-1 KO	1	Mito. ROS Mn-SOD GPx Catalase		SN	↑ ←→ ←→ ←→	(Guzman et al., 2010)
DJ-1 KO	4-6	Mitochondrial ROS		Entire brain	↑	(Irrcher et al., 2010)
LRRK2 KO	20	Protein oxidation Oxidative damage Autophagy-lysosomal pathway		Brain, Kidney	↑ ↑ ↓	(Tong et al., 2010)
[G2019S] LRRK2	2-4	Mitochondrial and autophagic abnormalities		Cortex	↑	(Ramonet et al., 2011)
Parkin KO	22	Protein oxidation		Striatum, cortex	←→	(Perez et al., 2005)
Parkin KO	11	Total GSH GSSH		Striatum	↑ ←→	(Itier et al., 2003)
Parkin KO	8 3,6,18 5-12 3, 18-20	Peroxiredoxin 1 Peroxiredoxin 2 Peroxiredoxin 6 Lactoylglutathione lyase 4-HNE Total antioxidant capacity Protein oxidation		Ventral midbrain Brain Serum Entire brain	↓ ↓ ↓ ↓ ←→3-6,↑18mo ↓ ←→3,↑18-20mo.	(Palacino et al., 2004)
Parkin KO	2,12	Mitochondrial aconitase GSH S-transferase P2 Carbonyl reductase 1 Lactoylglutathione lyase Thioredoxin reductase Protein oxidation		Striatum and Cortex	↑ cortex 2mo. ↓ both 2mo. ↑ striatum 2mo ↑ striatum 2mo ↑ striatum 2mo ←→	(Periquet et al., 2005)
Parkin KO	2,24	Total GSH GSH reductase activity GPx activity Catalase activity		Striatum, midbrain, limbic system	↑2,↓24mo. ←→ (w/in KO, ↑24 vs 2 mo) ←→ (w/in KO, ↑24 vs 2 mo) ←→	(Rodriguez-Navarro et al., 2007)
Parkin KO	19-21	Nitrotyrosine Nitrated α-synuclein		SN	↑ Only KO stain +	(Lu et al., 2009)
PINK-1 KO	3-4 24 22-24	Lipid peroxidation Protein oxidation 4-HNE Mitochondrial ROS Mn-SOD CuZu-SOD Catalase G6-PDH		Striatum SN Striatum	←→ ←→ ←→ ←→ ←→ ←→ ←→ ←→	(Gautier et al., 2008)
PINK-1 KO	2	Mitochondrial ROS			←→	(Akundi et al., 2011)
PINK-1 KO	4	GSH synthetase Peroxiredoxin 1 Protein oxidation		Striatum, midbrain, cortex	↑ striatum+cortex ↑ cortex ←→	(Diedrich et al., 2011)
PINK-1 KO	2-3	4-HAE 8-OHdG GSH/GSSG SOD activity Aconitase activity		Left ventricle	↑ ↑ ↓ ↓ ↓	(Billia et al., 2011)
[A53T]α-	12	iNOS, nitrite	TNFα	SN,	↑	(Gao et al., 2008)

synuclein tg		Superoxide Synuclein aggregates		striatum	↑ ↑	
[A53T]α-synuclein tg	2-7.5					(George et al., 2010)
[A53T]α-synuclein tg	12	Endoplasmic reticulum stress Abnormal unfolded protein response		SN, Striatum, Cortex, Brain stem, Spinal cord	↑ ↑	(Colla et al., 2012)
[A30P]α-synuclein Tg	6-14	Oxidized proteins		Whole brain	↑	(Poon et al., 2005)
[E46K]α-synuclein Tg	2-19	Protein aggregates		SN, striatum	↑ ↑	(Emmer et al., 2011)
α-synuclein 119	10	striatal dopamine		SN, striatum	↓	(Daher et al., 2009)

Table 3: Evidence for Oxidative Stress in Genetic Mouse models of PD

↑ represents increased levels of expression, ↓ represents decreased levels of expression and ←→ represents no difference in levels of expression.

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Model	Age (month)	Treatment	Marker of ox. stress	Marker of inflam.	Sampled region	Outcome vs control (neurodegeneration sensitivity)	Reference
DJ-1 KO	2-3	MPTP			SN, striatum	↑	(Kim et al., 2005)
DJ-1 KO	3-4	MPTP			Striatum	↑	(Manning-Bog et al., 2007)
DJ-1 Tg	Not reported	MPTP			SN	↓	(Paterna et al., 2007)
LRRK2 KO	2-3	MPTP			SN, striatum	←→	(Andres-Mateos et al., 2009)
Parkin KO	3	6-OHDA METH			Striatum	←→ ←→	(Perez et al., 2005)
Parkin Tg	Not reported	MPTP			SN	↓	(Paterna et al., 2007)
PINK-1 KO	24	Paraquat 6-OHDA DA Rotenone	Mitochondrial ROS		Striatum, cortex	←→	(Gautier et al., 2008)
PINK-1 KO	6	LPS		IL-1β, IL-12, IL-10	Striatum	↑	(Akundi et al., 2011)
PINK-1 KD	20	Paraquat			SN, striatum	↑	(Zhou et al., 2011)
PINK-1 Tg	2-3	MPTP			SN	↓	(Haque et al., 2008)
α-synuclein Tg	11-13	MPTP	Enlarged mitochondria		SN	↑	(Song et al., 2004)
[A30P]α-synuclein Tg	Not reported	MPTP			SN	←→	(Rathke-Hartlieb et al., 2001)
[A30P]α-synuclein Tg	3-8	MPTP Rotenone			SN SN	↑ ←→	(Nieto et al., 2006)
[A53T]α-synuclein tg	2	MPTP			SN	←→	(Dong et al., 2002)
α-synuclein KO	2	MPTP			SN, striatum	↓	(Dauer et al., 2002)

Table 4: "Two Hit" models of PD

"Two Hit" models of PD utilizing toxins with genetic mouse models have confirmed a pro-inflammatory response. ↑ represents increased sensitivity to neuronal degeneration when compared to the control, ↓ represents decreased sensitivity to neuronal degeneration when compared to the control and ←→ represents no difference in neuronal degeneration when compared to the control.

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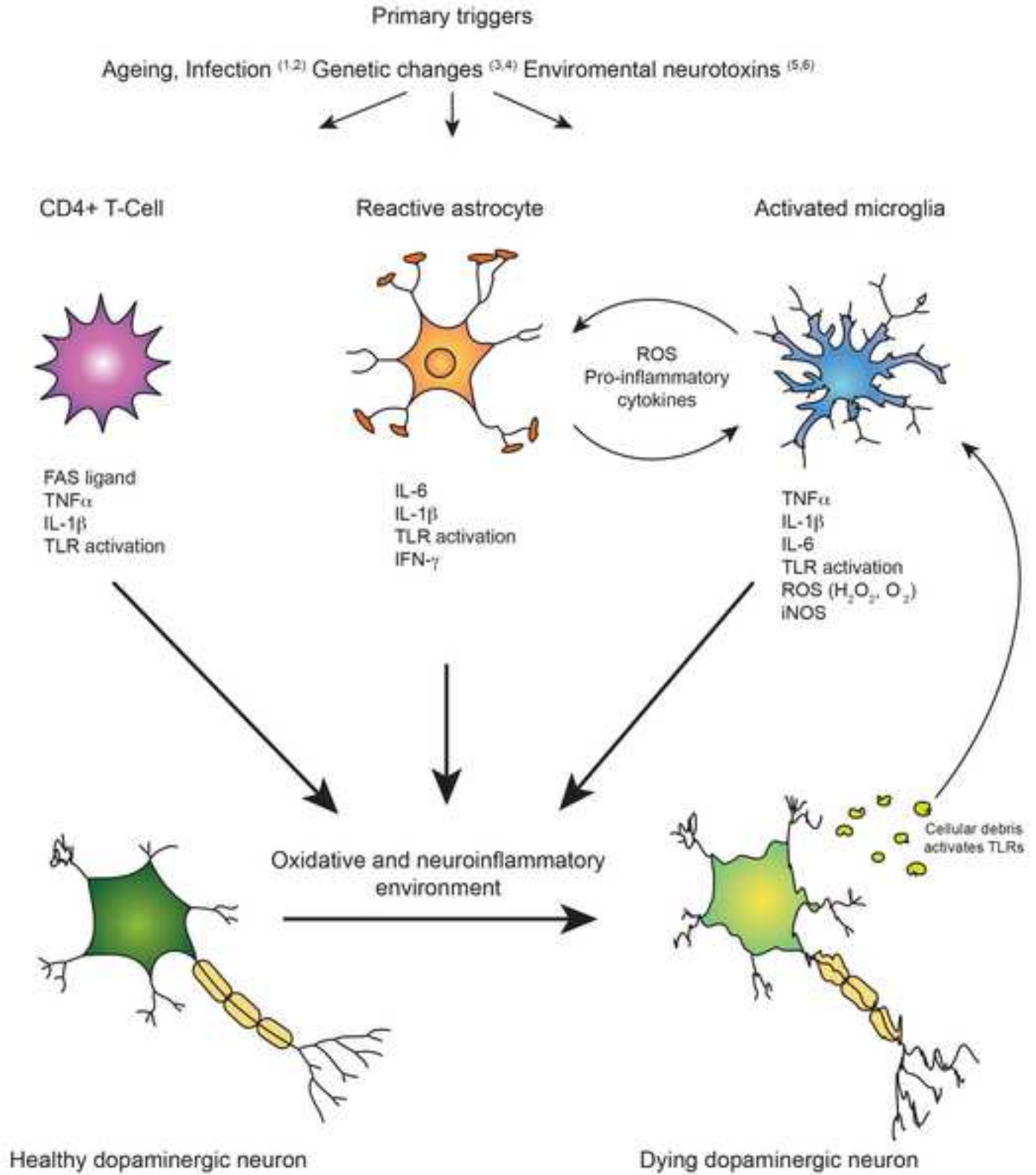
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Figure Legend

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Figure 1: The neuroinflammatory and oxidative stress response in PD. Primary triggers including exposure to environmental neurotoxins, brain injury or infection and genetic changes may converge in a complex interplay to induce dopaminergic neuronal degeneration. The resultant neuroinflammatory response may occur as a result of oxidative stress mechanisms within CNS cells and from components of the adaptive immune response with the recruitment of peripheral T cells through a compromised BBB. Activation of the innate immune systems resident CNS microglia and astrocytes further enhances the increased production of pro-inflammatory cytokines (TNFα, IL-1, IL-6), RNS and ROS creating a self-sustaining inflammatory environment within the SN. This can then have detrimental effects on healthy neuron populations, resulting in an amplified self-perpetuating cycle of microglial activation, reactive astrocytes and TLR activation leading to neuroinflammation and ROS-driven toxicity within the brain. [1]Bennett DA, et al., (1996) [2]Perry VH, et al., (2007) [3]Valente EM, et al., (2004) [4]Zimprich A, et al., (2004) [5]Langston JW, et al., (1984) [6]Higgins DS, Jr. and Greenamyre, JT (1996).

Figure 1.tiff

Primary triggers

Ageing, Infection [1,2] Genetic changes [3,4] Enviromental neurotoxins [5,6]

CD4+ T-Cell

Reactive astrocyte

Activated microglia

ROS
Pro-inflammatory
cytokines

FAS ligand
TNFα
IL-1β
TLR activation

IL-6
IL-1β
TLR activation
IFN-γ

TNFα
IL-1β
IL-6
TLR activation
ROS (H_2O_2, O_2)
iNOS

Oxidative and neuroinflammatory
environment

Cellular debris
activates TLRs

Healthy dopaminergic neuron

Dying dopaminergic neuron

- ➢ Neuroinflammation and oxidative stress have been implicated in neuronal loss in PD
- ➢ A pro-inflammatory response has been identified in human PD patients
- ➢ Genetic and toxin-based animal models have allowed a greater understanding of PD
- ➢ Oxidative stress and neuroinflammation have been confirmed in mouse PD models

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