2019

Mitochondrial dysfunction in C. elegans model of Parkinson's disease

BOSTON UNIVERSITY

SCHOOL OF MEDICINE

Thesis

MITOCHONDRIAL DYSFUNCTION IN *C. ELEGANS* MODEL OF PARKINSON'S DISEASE

by

SHIVALI MUKERJI

B.S., University of Rochester, 2017.

Submitted in partial fulfillment of the

requirements for the degree of

Master of Science

2019

Approved by

First Reader _____

Mina Moussavi, Ph.D.
Assistant Professor of Physiology and Biophysics

Second Reader _____

Gwynneth Offner, Ph.D.
Associate Professor of Medicine

ACKNOWLEDGMENTS

I would like to thank Dr. Gwyneth Offner and Dr. Mina Moussavi for their advice and guidance not only in writing this literature thesis but also in my graduate school courses and endeavors.

MITOCHONDRIAL DYSFUNCTION IN *C. ELEGANS* MODEL OF PARKINSON'S DISEASE

SHIVALI MUKERJI

ABSTRACT

Parkinson's disease (PD) is a devastating neurodegenerative disease and the second most prevalent after Alzheimer's disease. The most characteristic hallmark of Parkinson's is the presence of Lewy Bodies, clumps of aggregated α-synuclein protein, in the Substantia Nigra. While much has been said and theorized about α-synuclein, mitochondrial dysregulation in neurons of Parkinson's patients is an equally important consideration due to the role that the mitochondria plays in supplying neurons with their energy needs through ATP. *C. elegans* is a non-vertebrate animal often used to study aging and neurodegenerative disease due to its simple, well characterized genome. This literature review aims to outline the genetic and some environmental factors that cause mitochondrial dysregulation leading to the progressive neurodegeneration witnessed in Parkinson's, as modeled in *C. elegans*. Through a select review of studies done on *C. elegans* homolog of genes associated with mitochondrial function, this review aims to elucidate the mechanism by which each mutation not only causes the deficits seen in PD on its own but also how it interacts with other genes to worsen or alleviate symptoms. Ultimately, understanding these pathways and mechanism will be crucial to discovering and creating new therapeutic treatments and targets.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

LIST OF ABBREVIATIONS

AADC…….L-amino acid decarboxylase

ADE…….....Anterior deirid neurons

ALD.DH…….....Aldehyde Dehydrogenase

AR-JP……...……..Autosomal Recessive Juvenile Parkinsonism

BBB……..…….....Blood Brain Barrier

C. elegans…….....*Caenorhabditis elegans*

CEP…….....Cephalic neurons

COMT…….....Cateochol-O-methyltransferase

DA……Dopamine

DAT……...……....Dopamine Transporter

D. melanogaster……...*Drosophila melanogaster*

ETC…….....Electron Transport Chain

GIRK……..…G protein activated inward rectifying potassium channels

GPi…….……...Globus Pallidus

GWAS……...…….Genome Wide Association Studies

IMM…….....Inner Mitochondrial Membrane

KD…….....Kinase Dead

KO…….....Knockout

L-DOPA……...L-3,4-dihydroxyphenylalanine/Levadopa

LRRK2…….....Leucine Rich Repeat Kinase 2

MAP…….....Microtubule associated protein

MAO…….....Monoamine Oxidase

MAUI……...……..Monoamine Reuptake Inhibitors

MPTP……..……..1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine

mtDNA…….....Mitochondrial DNA

ND's……Neurodegenerative Diseases

NT……..……..Neurotransmitters

PD……..... Parkinson's disease

PDE…….....Posterior deirid neurons

Pu…… Putamen

ROS…….....Reactive Oxygen Species

SNc……. Substantia Nigra

SSRI……...Selective serotonin reuptake inhibitor

Th…….……..Thalamus

TH…….……..Tyrosine Hydroxylase

VMAT……Vesicular Monoamine Transporter

INTRODUCTION

Research on neurodegenerative diseases (ND's) has been on the forefront of scientific inquiry due in part to their elusive mechanism of action and the devastating impacts they have on quality of life of those affected. Parkinson's disease (PD) is widely recognized as one of the most prevalent neurodegenerative diseases, second only to Alzheimer's Disease (AD), affecting more than four million people worldwide. The hallmark symptoms of PD are quite well known- bradykinesia, tremors, rigidity, and impaired posture and balance (Nass et al., 2015). While the motor symptoms characterize this disease, the non-motor symptoms like anxiety, depression, memory deficits, can be just as devastating (Grover et al., 2015). These symptoms together produce the syndrome of Parkinsonism, of which PD is the main cause, although Parkinsonism can be neurodegenerative or non-neurodegenerative (Shulman et al., 2011). Since ageing is a significant risk factor for neurodegenerative diseases, finding therapies for ND's has become increasingly urgent due to a rapidly ageing population in post industrialized countries like the U.S. However, while PD is a well-studied disease with treatments like L-DOPA (described below) that have been prescribed for decades to manage the symptoms, a complete remediation of all symptoms and underlying pathology that can stop the progression of the disease or reverse the effects has yet to be discovered. This is due to the fact that the exact molecular events leading to neurodegeneration is still unknown (Caldwell & Caldwell, 2008).

Hallmark of Parkinson's disease

In 1817, James Parkinson, a British physician, published his study "paralysis agitans" which described a cluster of symptoms among six patients. The distinctive loss of dopaminergic neurons from the Substantia Nigra as well as the formation of protein aggregates, called Lewy Bodies and dystrophic neurites, that is observed in PD, had been observed as early as 1912 (Shulam et al., 2011). Figure 1 is a simplified illustration of the brain areas and pathways involved in the dopaminergic signaling in a healthy vs normal individual. The Substantia Nigra (SNc) provides excitatory dopaminergic input to the putamen (Pu). The Pu inhibits the Globus Pallidus (GPi) which then inhibits Thalamus (Th) which in turn send excitatory signals to the motor cortex. In patients with PD (left panel Fig 1) this signaling is attenuated (shown through dotted lines) (Shulman et al., 2011).

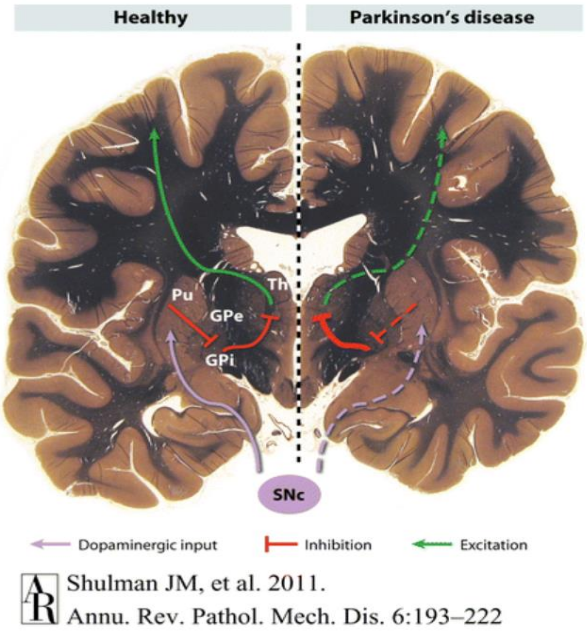


Fig 1: This simplified illustration shows the neuronal circuits involved in basal ganglia, thalamus and cortex (left) and degeneration in these pathways believed to occur in PD patients (right). Taken from Shulman et al., 2011.

While PD can be genetic/familial inherited, only 10% of PD cases are actually due to familial factors and the majority of cases are sporadic/environmentally linked (Warner & Schapira, 2003). Toxins such as 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) (Langston et al., 1983) and pesticides like paraquat, maneb (Thiruchelvam et al., 2013), and rotenone (Tanner, 1992) have all been shown to cause PD. Occupational hazards like exposure to heavy metals like manganese has also been linked to the environmental causes of PD. However, the majority of PD cases are still of unknown/ sporadic cause (Tanner, 1992). It has been observed that the clinical symptoms presented in many familial forms of PD are similar to the clinical symptoms presented in

many sporadic forms of PD (Guo, 2012). Genome Wide Association Studies (GWAS) have also shown that many of the polymorphisms associated with familial PD also contribute to sporadic PD (Simon-Sanchez et al., 2009). By 1997, α-synuclein was found to be the culprit and the genetic cause for familial inherited PD and the main component of Lewy Bodies (Spillantini et al., 1997). Soon, eight other gene products were identified including PARK2/*Parkin*, PARK5/*UCHL-1*, PARK9/*ATP13A2*, PARK8/*LRRK2*, PARK11/*GIGYF2*, PARK6/*PINK1*, PARK7/*DJ1* and PARK13/*HTRA2* (Dawson & Dawson, 2003). Mutations in these genes along with their pattern of inheritance have been detailed in Table 1. For example, the PARK2/*Parkin* mutation leads to autosomal recessive juvenile Parkinsonism (AR-JP) (Bonifati et al., 2002).

Table 1
Loci and genes linked to familial PD

Locus	Chromosomal location	Gene	Mode of inheritance
PARK1	4q21.3	*α-Synuclein*	Autosomal dominant
PARK2	6q25.2-27	*Parkin*	Autosomal recessive
PARK3	2p13	Unknown	Autosomal dominant
PARK4	4p15	Unknown	Autosomal dominant
PARK5	4p14	*UCH-L1*	Autosomal dominant
PARK6	1p35-p36	Unknown	Autosomal recessive
PARK7	1p36	*DJ-1*	Autosomal recessive
PARK8	12p11.2-q13.1	Unknown	Autosomal dominant
PARK9	1p36	Unknown	Autosomal recessive (Kufor-Rakeb syndrome)
PARK10	1p32	Unknown	Late-onset susceptibility gene

Table 1: Common genetic mutations associated with familial PD and mode of inheritance. Figure taken from Dawson & Dawson, 2003.

Mechanism of Dopamine Action

The neurodegeneration observed in PD is not just limited to dopaminergic neurons (although this is the system it largely affects) but also affects serotonergic and noradrenergic neurons (Scatton et al., 1983). In the nigrostriatal dopaminergic neurons, dietary phenylalanine is converted to tyrosine, and along with blood borne tyrosine taken up by amino acid transporters in dopaminergic neurons, tyrosine is converted to L-3,4-dihydroxyphenylalanine (Levodopa or L-Dopa) by tyrosine hydroxylase. This is the rate limiting step in the biosynthesis of dopamine (DA). L-amino acid decarboxylase (AADC) catalyzed the conversion of L-DOPA to DA, (Elsworth & Roth, 1997). These reactions are summarized in Figure 2.

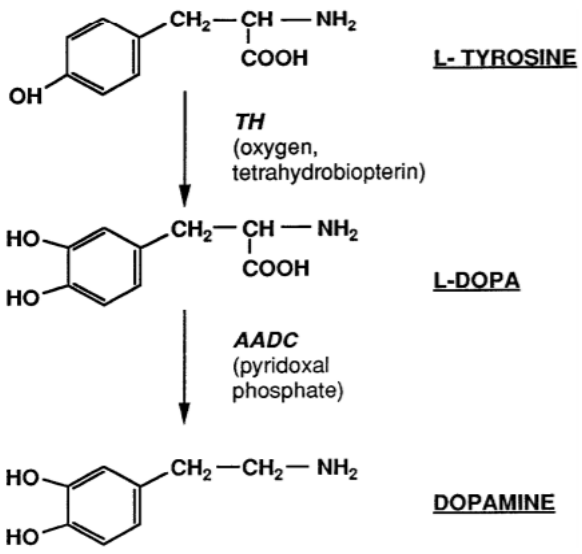


Fig 2: Biosynthesis of DA from Tyrosine. TH: Tyrosine Hydroxylase, AADC= L-aromatic amino acid decarboxylase. Cofactors given in parasynthesis. Taken from Elsworth & Roth, 1997.

Classical Neurotransmitters (NT) are stored in secretory vesicles by secondary active transporters known as vesicular monoamine transporter (VMAT). VMAT2, a type of VMAT, is the transporter responsible for storing all monoamines into small synaptic and dense core secretory vesicles- catecholamines, serotonin, and acetylcholine. A proton gradient is used to transport NT against their concentration gradient (Gasnier, 2000). Following an action potential, an influx of calcium provides the stimulus needed for fusion of vesicles to the neuronal membrane (Elsworth & Roth, 1997). DA is then released in a regulated manner from presynaptic axon terminals in striatum or presynaptic dendrites in Substantia Nigra (a component of the midbrain). Once in the extracellular space, this dopamine will then interact with receptors on neurons or glial cells. This signaling is terminated by reducing the amount of DA in the extracellular space using Na^+ and Cl^- dependent dopamine reuptake carriers (Nirenberg et al., 1996). The presence of a dopamine transporter (DAT) was first confirmed in molecular cloning studies done in rat models. It was also shown that these transporters also had the ability to uptake DA neurotoxins, such as cocaine and other psychostimulant drugs (Shimada et al., 1991). Most importantly, DAT only exists on dopaminergic neurons and DA can only be re-up taken by DA neurons (Nirenberg et al., 1996). This is in contrast to other neurotransmitter (NT) systems like glutamate (Rice et al., 2011). The DA leftover in the cleft is metabolized by monoamine oxidase (MAO-A, MAO-B), aldehyde dehydrogenase (ALD.DH), and catechol-O-methyltransferase (COMT) into homovanillic acid through various pathways.

The majority of DA receptors are located on non-DA neurons however some DA receptors are also present on DA neurons themselves. The latter type are also called autoreceptors and provide negative feedback to inhibit DA neuron firing and synthesis, release, and uptake of DA (Ford, 2014). These autoreceptors are usually of the D2-subtype of DA receptors which are inhibitory and activate G protein activated inward rectifying potassium channels (GIRK). Autoreceptors also modulate DA neuron activity indirectly through control of tyrosine hydroxylase expression and plasma membrane DAT to modulate DA transmission (Beaulieu & Gainetdinov, 2011). Overall, the majority of the auto feedback inhibition of DA signaling is mediated through the D2-subtype of DA receptors (Ford, 2014).

Current Treatments for PD

The most prescribed treatments for PD currently is dopamine replacement therapy with L-3,4-dihydroxyphenylalanine (Levodopa or L-DOPA). This is given along with an aromatic L-amino acid decarboxylase (AADC) inhibitor such as benserazide or carbidopa to prevent nausea and vomiting associated with administration of plain L-DOPA as well as to increase the bioavailability of L-DOPA (Fahn et al., 1971). Once administered, orally or intravenously, L-DOPA undergoes decarboxylation by AADC and is converted to DA in the periphery. However, it is important to note that this peripheral DA cannot enter the brain due to the blood brain barrier (BBB). Carbidopa acts by inhibiting peripheral AADC and thus reducing the conversion of L-DOPA to dopamine in the periphery and increasing the bioavailability of pure L-DOPA. L-DOPA is able to cross

the BBB and is converted to DA inside dopaminergic neurons through the action of AADC present in the neuron. Vesicular Monoamine Transporters (VMAT) then stores this newly made DA into vesicles, to be released into the synaptic cleft under the appropriate stimulation (Nishijima & Tomiyama, 2016). Figure 3 depicts the pathway that L-DOPA injected in the periphery takes to cross the BBB and act on the CNS. By increasing the availability of DA in the striatum as well as extrastriate areas of patients with PD, L-DOPA treatment acts by counteracting the loss of dopaminergic neurons in PD (Nishijima & Tomiyama, 2016).

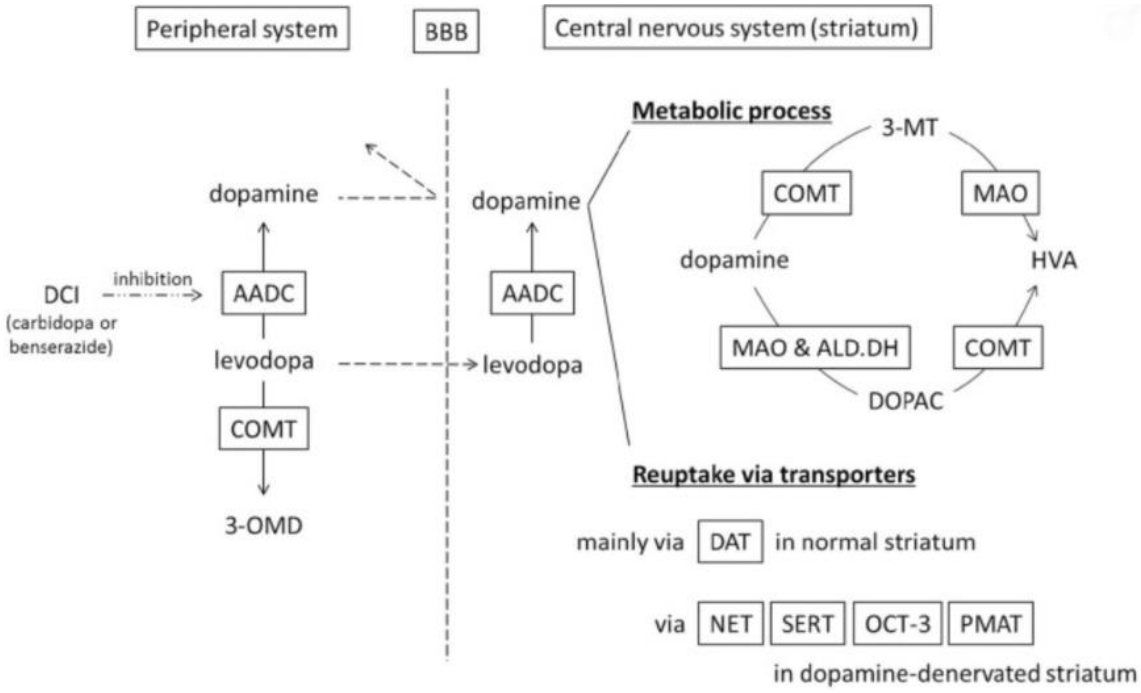


Fig 3: Illustration of the mechanism of action of L-DOPA treatment given alongside AADC inhibitor (carbidopa or benserazide) to inhibit the conversion of L-DOPA to dopamine in the periphery and increasing L-DOPA bioavailability. L-DOPA crosses the

BBB and helps combat the loss of dopaminergic neurons and DA signaling in patients affected by PD. Taken from Nishijima & Tomiyama, 2016.

It has also been observed that when dopaminergic neurons undergo significant degeneration, serotonergic neurons take on the role of converting exogenous levodopa to DA, storing, and releasing the newly made DA (Arai et al., 1994, 1995). However, as mentioned before, DAT only exists in dopaminergic neurons and serotonergic neurons cannot re-uptake DA once it is released in the cleft. Moreover, serotonergic neurons do not contain D2 receptors and thus negative feedback of DA synthesis and release does not happen and this process becomes unregulated (Nishijima & Tomiyama, 2016). Pulsatile fluctuations of extracellular DA, due to unregulated release of DA, leads to many of the levodopa induced motor complications such as dyskinesia (Olanow & Obeso, 2000). Figure 4 (A) depicts the normal dopaminergic signaling pathway while Figure 4 (B) shows the alternative serotonergic pathway for DA release that occurs in PD patients who have extensive dopaminergic neuron loss. Another major problem with L-DOPA is that chronic administration of the drug is hypothesized to cause loss of drug efficacy over time. This phenomenon is known as tachyphylaxis, and has been shown to decrease the efficiency of alternative DA synthesis from serotonergic neurons, which themselves undergo degradation over time (Navailles et al., 2011).

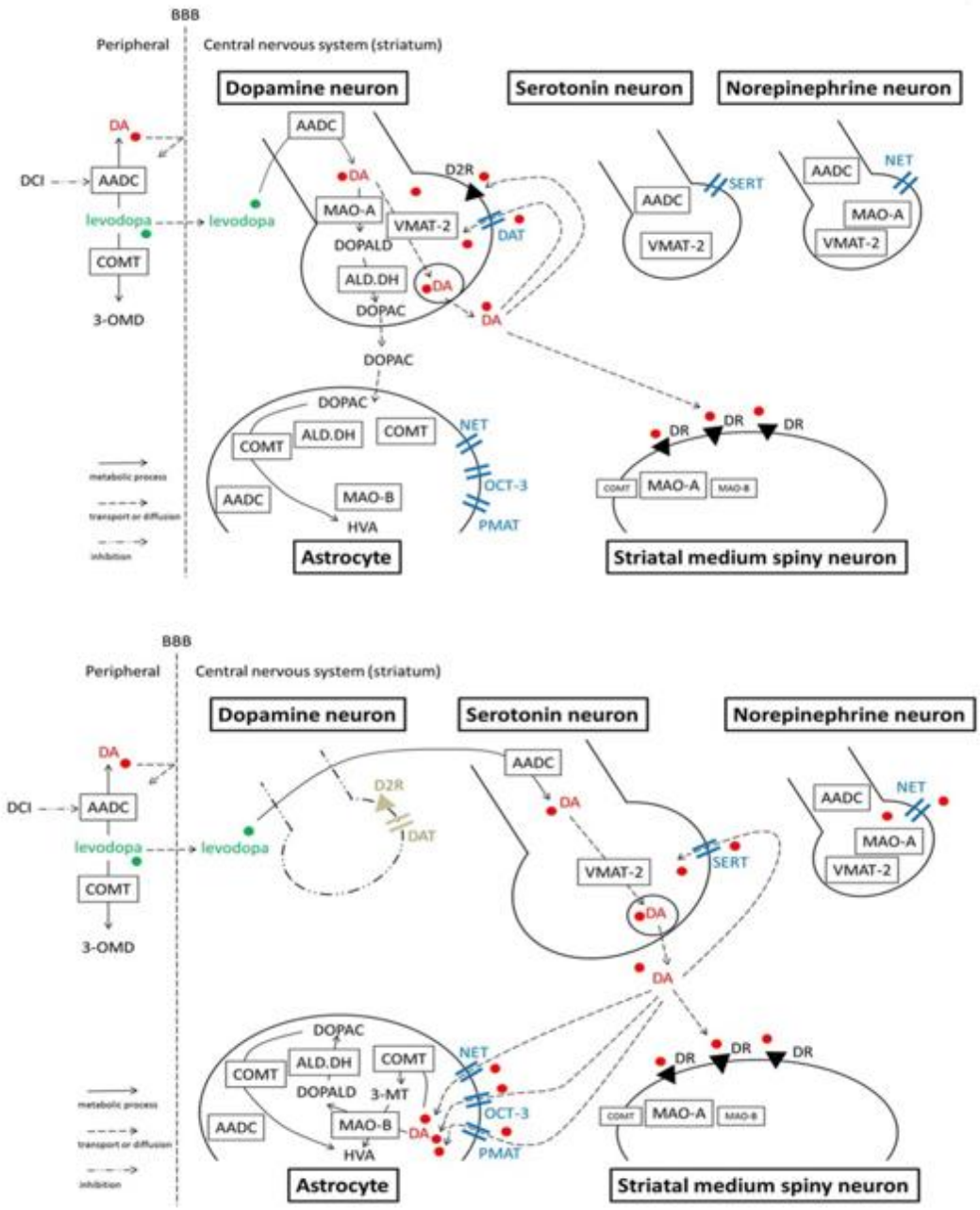


Fig 4 (A): Pathway of normal dopaminergic neuron signaling in the striatum. Taken from Nishijima & Tomiyama, 2016.

Fig 4 (B): Alternative pathway of dopaminergic signaling through serotonergic neurons in a dopamine deprived striatum. Figure depicts the absence of DAT as well as D2-subtype autoreceptors in serotonergic neurons. Taken from Nishijima & Tomiyama, 2016.

Another treatment route for patients with PD has been in the use of Monoamine Reuptake Inhibitors (MAUI's) that act by inhibiting the action of monoamine transporters and hence increasing the amount of NT available in the synaptic cleft. This could lead to alleviation of both motor and non-motor symptoms. Studies on the efficacy of selective serotonin reuptake inhibitors (SSRIs), that are prescribed largely for depression, have shown mixed results in treating the motor symptoms associated with PD but several studies have validated their ability to alleviate non motor symptoms such as anxiety and apathy (Huot et al., 2015). Similarly, researchers comparing various studies done on toxin induced PD animal models showed that DAT inhibitors exerted anti-Parkinsonian effects when given by themselves or when given along with L-DOPA but did not enhance the effects of the L-DOPA treatment. Overall, while the use of MAUI's to treat PD has extended as far back as the 1930's and they are used extensively to treat the non-motor symptoms of PD such as depression, their application beyond this has not been proven in humans and no MAUI has shown its effectiveness in alleviating any symptoms apart from depression in Phase III clinical trials (Huot et al., 2015). Furthermore the same tachyphylaxis associated with L-DOPA treatment is also experienced with MAUI's over time.

Overall, while certain symptoms of Parkinson's like depression or early motor symptoms can be controlled with the use of pharmaceuticals such as MAUI or L-DOPA, none of these treatments can provide a full resolution of symptoms or even a reversal of the neurodegeneration seen in PD. Furthermore, many of these treatments come with their own side-effects such as dyskinesia that is experienced with L-DOPA. This creates a demand for viable animal models to study various treatments and drugs before they can be tested out safely in human beings.

Models of PD

Human cell based studies have limited primary tissue availability and while post-mortem studies can correct for this, it fails to follow the progression of the disease starting from the initial pre-symptomatic stages (Guo, 2012). Genetic analysis and screening of the human genome is invaluable for testing of therapeutic drugs and neuroprotective gene products. But vertebrate as well as invertebrate models of PD help overcome the shortcomings of human cell based studies and facilitate research and development. Mammalian models are hugely beneficial to researchers due to easily identifiable features like the loss of DA neurons (Thiele et al., 2012), replication of motor movement deficits involved in PD- tremors, bradykinesia, and muscle rigidity can be easily studied in rodent models (Lees et al., 2009), as well as non-motor deficits like depression and cognitive decline (Taylor et al., 2010). However, rodent models can be difficult to work with due to the significant time and financial investment required to develop complex procedures (Maulik et al., 2017).

Currently there are two types of primary experimental models used to study PD- genetic and toxin based- and are most beneficial when used in conjunction (Jagmag et al., 2016). It has been shown that since many rodent models rely on the neurotoxin based models of PD, they are often unable to mark the gradual neurodegeneration and gross morphological abnormalities that is characteristic of PD (Riberio et al., 2013). The biggest drawback of the toxin model is that when toxins like MPTP, rotenone, or paraquat, are induced into the system they cause degeneration of 70-80% of DA neurons and hence miss the critical window of Lewy Body (seen universally in PD patients) formation and development that is so critical to age related DA neuronal loss in human patients (Schirinzi et al., 2016).

All of these reasons lead to the search of other viable animal models. This is where invertebrate models such as *Drosophila melanogaster* and *Caenorhabditis elegans* can prove invaluable due to their cost effectiveness and studies done with these models can then be replicated on mammalian models. Both of these models contain many of the important familial PD homologs except the gene for a crucial protein involved in PD known as α-synuclein (Jagmag et al., 2016). The fly *D. melanogaster* contains homologs for *DJ1, PINK1, Parkin, LRRK2,* and *VPS35* and was crucial in elucidating the role of mitochondrial dysregulation in the progression of PD that is discussed below (Venderova et al., 2019). Finally, *D. melanogaster* shows deficits in multiple systems and not just DA neuron degeneration which is analogous to the progression of PD in humans (Guo, 2012).

Significance of *C. elegans* model in PD research

C. elegans is another invertebrate that has been extremely useful in the study of PD. *C. elegans* make for a useful model to study experimentally due to their transparency, microscopic size, rapid life cycle, and well annotated genome. However, there are other characteristics that set them apart for research use- such as the presence of two sexes- hermaphrodites (modified females) and males. Hermaphrodites are of significance since they can self-fertilize using the stored sperm created by their gonads. This means one single animal can produce 300 progeny and populate an entire agar plate on their own. However the evolutionary advantage that males possess is that when mated with a hermaphrodite, the hermaphrodites can then produce up to 1000 progeny, which also makes the hermaphrodite produced sperm a limiting factor in fertilization (Corsi et al., 2015). Another unusual feature of *C. elegans* is the ability of the worms to arrest development in the larval stage, called *dauer*, when environmental conditions are stressful (lack of nutrition, hypoxia etc.). In laboratory practice, these *dauer* stage animals can last from a month to six months at 15 degrees as stocks that do not require many nutrients (Corsi et al., 2015). Most importantly these animals live in the soil and must sense their environment in order to survive. The presence of the *dauer* stage, mating with a male for increasing the number of progeny, and general colliding against soil particles and other nematodes are some of the reasons why mobility and sensorimotor neurons play a significant role in the otherwise simple organism *C. elegans*. The transparency of the worms makes it easy to study their development and function at the level of a single cell (Corsi et al., 2015). Additionally, their transparency also confers another advantage

as their neurons can be easily visualized using GFP staining, which is a fluorescent protein that was originally experimentally demonstrated in *C. elegans* and even won its discoverer, Martin Chalfie, the 2008 Nobel Prize in Chemistry (Harrington et al., 2010). Furthermore, the *C. elegans* genome has been completely sequenced and 80% of the genes have been found to have human homologs (Consortium C.E.S, 1998).

Unlike the human body, which contains thousands of dopaminergic neurons in the brain, the hermaphrodite *C. elegans* only have eight dopaminergic neurons- all of which are believed to be involved with mechanosensation. This confers an advantage to the *C. elegans* as an invertebrate model for studying PD since it makes for a simplistic model that can be easily manipulated. The eight DA neurons are split accordingly- four cephalic neurons, two anterior deirid neurons, and two posterior deirid. Figure 5 provides an illustration of the position of these neurons with respect to the worm's body.

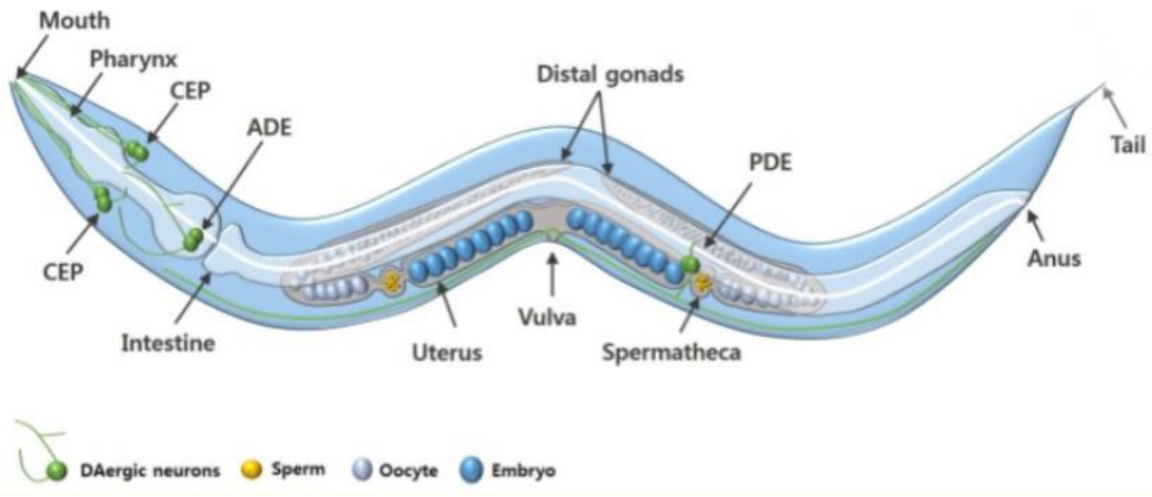


Fig 5: Illustration of a hermaphrodite adult *C. elegans* depicting all eight DA neurons - four cephalic neurons (CEP), two anterior deirid neurons (ADE), two posterior deirid (PDE). Figure taken from Chege & McColl, 2014.

It is also important to note that male *C. elegans* have six additional DA neurons located in the tail, which are thought to be involved in mating behaviors (Sulston et al., 1975). The eight other neurons are thought to be involved in basal motor activity, habituation, egg-laying, and defection. These neurons even contain many of the same enzymes that are found in mammalian DA neurons like tyrosine hydroxylase, monoamine transferase, MAO, and COMT as well as DAT (Nass, et al., 2008). Furthermore, *C. elegans,* like *D. melanogaster*, have homologs for many of the same genes involved with familial PD in humans like *PINK1, PARK, DJ-1* and *LRRK2* (Chege and McColl, 2014). But just like *D. melanogaster,* the *C. elegans* does not have a homolog for α-synuclein gene but it can be ectopically expressed in the organism. This would involve artificially inducing the gene expression to determine the function of the gene in question. Fig. 6 (A)

depicts the eight DA neurons tagged using a GFP maker in a live *C. elegans* adult hermaphrodite (Nass et al., 2002). This allows researchers to study the pathogenesis of PD or any other ND in real time and follow the progression of neurodegeneration and the other microscopic markers for PD. In Fig. 6(B), researchers ectopically induced the expression of the α-synuclein A53T isoform in *C. elegans.* Compared to wild type, these genetically manipulated worms show reduced fluorescence and loss of cell body as well as axonal processes (Nass et al., 2007).

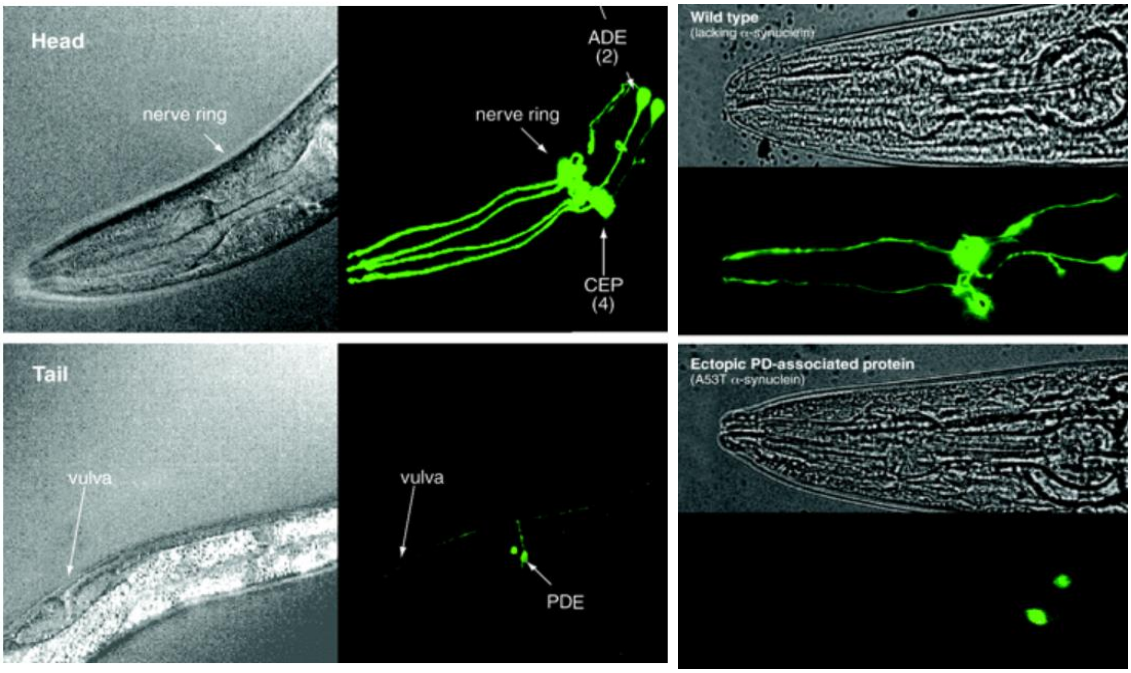


Figure 6(A): Left panel-The eight dopaminergic neurons of a live hermaphrodite adult *C. elegans* tagged with GFP and imaged using confocal microscopy. Taken from Nass et al., 2002.

Figure 6(B): Right panel- Expressing human a-synuclein isoform A53T causes *C. elegans* DA neurons to degenerate. Wild type (top right panel) neurons show GFP fluorescence throughout the cell body but in the A53T expressing isoform (bottom right panel) shows loss of cell body and processes. Only the ADE pair of neurons is still expressing fluorescence tagging. Taken from Nass et al., 2007.

While *C. elegans* makes for a suitable model for neurodegenerative diseases, these organisms also have some shortcomings as a model. The most apparent flaw being that due to their simplistic nature. By only having eight dopaminergic neurons, it is often difficult to account for the complicated and vast network of neurons in mammals. However, this can be accounted for by replicating these studies in higher vertebrates like rodents and other mammalian models including non-human models primate models. Additionally, *C. elegans* have a tough outer cuticle covering which makes it difficult to administer drugs and other therapeutic compounds since it is difficult for these compounds to cross the barrier. This can also be corrected by administering drugs at a higher concentration and with increased dose regimes (Chen et al., 2015). Overall, despite these complications, *C. elegans* still makes a viable model for toxin and genetically modified models of neurodegenerative diseases and research done on these models can be easily translated to vertebrate research.

PD AND THEORIES OF MECHANISM

Since the discovery of the various PARK proteins discussed earlier, researchers were able to confirm specific cellular defects that are associated with these proteins (Shulman et al., 2011). For example, PARK1 or α-synuclein is, discussed below, is associated with proper synaptic functioning. PARK2/*Parkin*, PARK9/*ATP13A2*, and PARK5/*UCHL-1* were found to be associated with protein degradation whereas PARK8/*LRRK2* and PARK11/*GIGYF2* are involved in signal transduction. Finally, PARK8/*LRRK2* and PARK11/*GIGYF2* were found to be protective against mitochondrial/oxidative stress (Shulman et al., 2011). While familial PD makes up for a minority of all PD cases, with the majority being sporadic PD, these proteins and changes in their normal functioning are crucial in understanding the pathogenesis of PD. Several important pathways have been discovered and two of significances have been highlighted below.

α-synuclein

Mutation in the gene producing α-synuclein was identified early on as a hallmark in the majority of familial PD cases. α-synuclein in particular, has been well-studied due to its presence in Lewy Bodies which is a pathological hallmark of PD. It is a 140 amino acid peptide with three domains encoded by the *SNCA* gene and found predominantly in the presynaptic terminals of dopaminergic neurons. The *SNCA* gene is highly expressed in the Substantia Nigra, hippocampus, neocortex, thalamus and cerebellum (Recchia et

al., 2004). These regions also happen to be the same ones that are the most affected by neurodegeneration in PD pathology. Several point mutations in the *SNCA* gene have been implicated in familial PD (Polymeropoulos et al., 1997). α-synuclein knockout (KO) mice have also been shown to have impaired spatial learning and working memory (Kokhan et al., 2012). Other studies have suggested that this protein negatively modulate DAT (Wersinger and Sindhu, 2003), leading to unregulated DA release and the resulting unbound DA can act as a neurotoxin (Offen et al., 1999), or that it is a microtubule associated protein (MAP) (Alim et al., 2002). As well studied as α-synuclein is, the exact function of this protein is actually unknown (Chege et al., 2014). While much research and many reviews have been written about the α-synuclein centric theory of protein aggregation, another pathway that deserves equal attention and works is conjugation with these other pathways is the mitochondrial dysregulation pathway.

Mitochondrial Dysregulation and Oxidative Stress

Mitochondrial dysregulation has been implicated in PD for some time but the first evidence came through substance users. It was found that exposure to an environmental toxin 1-methyl-4-phenyl-1,2,3,4-tetrahydropyridine (MPTP), caused the symptoms of Parkinsonism. The 1-methyl-4-phenylpyridinium ion (MPP+) was found to not only be an inhibitor of complex I, situated in the inner mitochondrial membrane (IMM), but also a substrate for DAT (Langston et al., 1983). This inhibition of complex I led to toxicity and death of DA neurons. Not only does it have this direct effect, but MPP+ toxicity through complex I inhibition was also shown to decrease ATP production, increase

oxidative stress as well as increase toxicity through increased DA release (Abou-Saleim et al., 2006). Even in post-mortem brain tissue of PD patients, a marked inhibition of complex I is seen in the cortex and Substantia Nigra (Krige et al., 1992). Research done in the *D. melanogaster* model was crucial to the discovery of a common pathway through which two PD genes, *PINK1* and *Parkin* act by maintaining mitochondrial integrity (Guo, 2012). In fact, many studies have also shown a correlation between α-synuclein aggregation in the mitochondria and oxidative stress in the pathogenesis of PD (Spillantini et al., 1997).

The agents of oxidative stress are thought to be reactive oxygen species (ROS) such as hydroxyl radical, superoxide, and hydrogen peroxide. The production of these oxidative species has been linked directly back to the mitochondria and enzymes within the electron transport chain, the most important of which are complex I and III (Lambert et al., 2009). Imbalance between ROS and antioxidants, which maintain homeostasis, in favor of ROS causes oxidative stress (Yan et al., 2013). In the brain, oxidative stress leads to neuronal loss. The mitochondria is responsible for 90% of ROS production since electrons can leak out of the electron transport chain and react with oxygen (Yan et al., 2013). Increased formation of mitochondrial ROS leads to damage of mitochondrial DNA (mtDNA), proteins, lipids, and redox signaling pathways (Lambert et al., 2009). These changes are often associated with the mitochondrial theory of aging as well as neurodegenerative changes but have been shown to have neuroprotective effects as well (Winklhofer & Haass, 2010). While deficiency of complex I in PD patients has been well observed, the cause of it is still not known. However, increased oxidative damage is seen

in animal models with toxin induced PD where the toxin, like rotenone, is a known inhibitor of complex I. So while it is known that there is increased oxidative damage due to mitochondrial dysregulation in patients with PD, and that this oxidative stress may be neuroprotective at first, the exact mechanism by which these changes lead to gradual neurodegeneration witnessed in PD is still unknown (Winklhofer & Haass, 2010).

The mitochondria plays a major role in neuronal survival and is the major energy producer in the brain, the most energy intensive organ in the body (Yan et al., 2013). This is because neurons have very limited capacity for glycolysis and so the majority of their energy needs are met by oxidative phosphorylation. ROS production, coupled with the short half-life of mtDNA, leads to accumulation of mutations in mtDNA that has been hypothesized to lead to decreased electron transport chain efficiency, reduced ATP production, and in turn higher ROS production, thus continuing the cycle of mitochondrial stress (Yan et al., 2013). Given the importance of mitochondrial dysregulation and oxidative stress in the progression of PD, the rest of this review will outline the past and current research on the genetic and toxin based models of PD in *C. elegans* that specifically cause the pathogenesis of PD through mitochondrial dysregulation and hence increased oxidative stress. This will help researchers identify potential future targets and other overlapping pathways that could all lead to improved understanding of the disease itself as well as targets of therapeutic interventions.

PUBLISHED STUDIES

Genetic Models for PD in *C. elegans*

As previously mentioned, familial PD is associated with mutations in eight genes and although these mutations are rare they are still of significance when trying to deduce the etiology of PD. Of these eight genes, four - *Parkin, DJ1, LRRK2* and *PINK1* are all related to mitochondrial function (Exner et al., 2007). *C. elegans* have homologs for a lot of these genes- for example: *lrk-1* is the *C. elegans* homolog for *LRRK2*. In the following section, we discuss three of these genes- *LRRK2, PINK1* and *Parkin*. Research into these genes utilizes two main principles (as observed in the following studies)- either the human gene or protein is expressed in *C. elegan*s strains or the homologous gene is mutated in the nematode to then study the effects of these alterations.

LRRK2

Mutations in leucine rich repeat kinase 2 (*LRRK2*) lead to the autosomal dominant form of PD and are associated with familial and late onset PD (Zimprich et al., 2004). *LRRK2* is a large multi domain protein with both a GTPase and kinase domain (Gandhi et al., 2009, Guo et al., 2006). The most common mutation is G2019S and is present is 30% of familial PD in the Ashkenazi Jewish populations (Ozelius et al., 2006). With respect to this particular mutation, G2019S, it has been found to cause the uncoupling of mitochondrial oxidative phosphorylation (Mortiboys et al., 2010). G2019S mutation also leads to increased *LRRK2* phosphorylation and kinase activity (Greggio et al., 2006).

LRKK2 is thought to be present in the cytoplasm and associated with mitochondrial, endoplasmic reticulum, and synaptic vesicle membranes (Biskup et al., 2006). While the exact function of *LRRK2* is still not known, its *C. elegans* homolog *lrk-1* exhibits some role in the polarized sorting of synaptic vesicles (Sakaguchi-Nakashima et al., 2007). Neurons are polarized cells with dendritic and axon/presynaptic end. Synaptic vesicle proteins are exclusively located in presynaptic regions and not in dendrites. In 2007, Sakaguchi-Nakashima et al., found that when *lrk-1* is deleted in *C. elegans*, synaptic vesicle proteins localize to both presynaptic and dendritic ends. This is an interesting result given that α-synuclein is also found on pre-synaptic ends of neurons and aggregate in Lewy Bodies, which is a pathological hallmark of PD. This led researchers to hypothesize that *LRRK2*/*lrk-1* must regulate synaptic vesicle protein localization in both the nematode as well as the human (Sakaguchi-Nakashima et al., 2007). However, a different study found that in a co-immunoprecipitation assay, *LRRK*2 interacts with *Parkin* but not α-synuclein or *DJ-1* (Smith et al., 2005). While this does not negate a possible pathway through which *LRRK2* and α-synuclein might interact, it does mean that other pathways or molecules might be involved in their interaction.

To elucidate the role of *LRRK2* mutations in neurodegeneration, Yao et al., in 2010 found that ectopic expression of LRRK2 proteins in *C. elegans* as well as two mutations of the LRRK2 protein- R1441C and G2019S- all demonstrate age dependent degeneration of DA neurons, behavioral deficits, and locomotor dysfunction (Fig 4) compared to a control GFP tagged strain. More importantly, the same study also found that when a GTPase binding mutation K1347A is introduced, the neurodegeneration is

reversed suggesting a role for the GTPase domain in the neurodegeneration seen in *LRRK2* mutations (Yao et al., 2010).

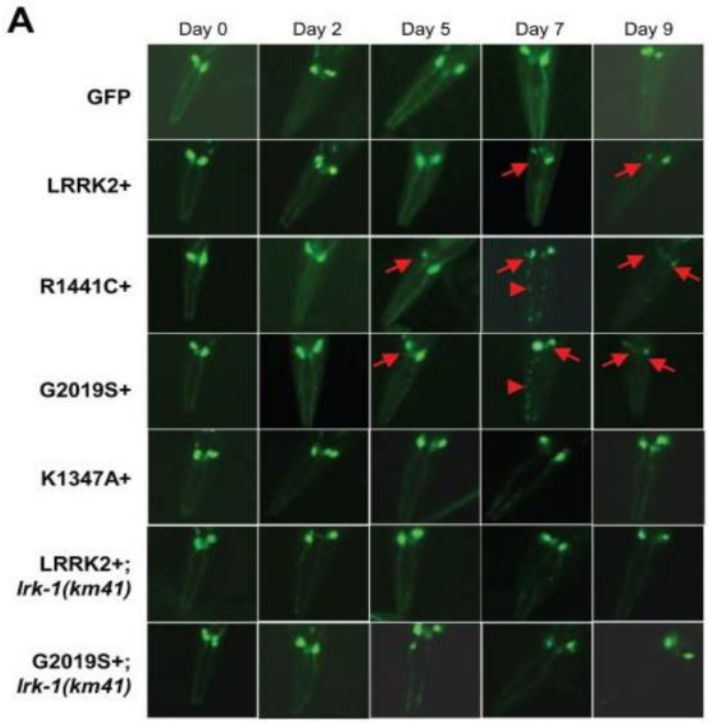


Fig 7: From top to bottom- Control GFP tagged *C. elegans* that shows intact DA neurons and axonal neuritis, but *LRKK2*, R1441C, G2019S, show loss of DA neurons (red arrow) and broken neuritis (arrowhead) during adulthood. K1347A provides protection. Taken from Yao et al., 2010

To elucidate the role that *LRRK2* may play in mitochondrial dysregulation, Saha et al., (2009) used age synchronized lines of young adult *C. elegans* of the following strains- wild type (WT) *LRRK2*, G2019S mutated strain, R1441C mutated strain and two kinase dead strains - KD and KD/R1441C. The KD strain is a kinase dead strain where

the *LRRK2* functionality is inhibited and behaves akin to a control. *C. elegans* were exposed to rotenone and paraquat, known oxidative stress and mitochondrial dysregulation agents. Figure 8(A) shows the survival rate of each of these strains. It is observed that the WT *LRRK2* as well as the KD/R1441C strains have significantly higher survival rates. This shows that WT *LRRK2* must have some type of neuroprotective role against mitochondrial stress. Additionally, since the KD R1441C strain also showed significantly higher survival rate, researchers hypothesize that perhaps the kinase domain is not important in conferring protection against rotenone but this is only the case for a double mutation since this high survival rate is not seen in only KD strain. More importantly, when the researchers knockdown endogenous *lrk-1*,through RNAi expression of a plasmid against *lrk-1*, exposing these *lrk-1* deficient *C. elegans* to rotenone causes sensitization of *C. elegans* to mitochondrial inhibition leading to significantly lower survival rates when compared to *C. elegans* with *lrk-1* intact (Fig 8(B)) (Saha et al., 2009). This result shows that the nematode's own homolog of *LRRK2, lrk-1*, also has a neuroprotective role when the nematode is under mitochondrial stress conditions.

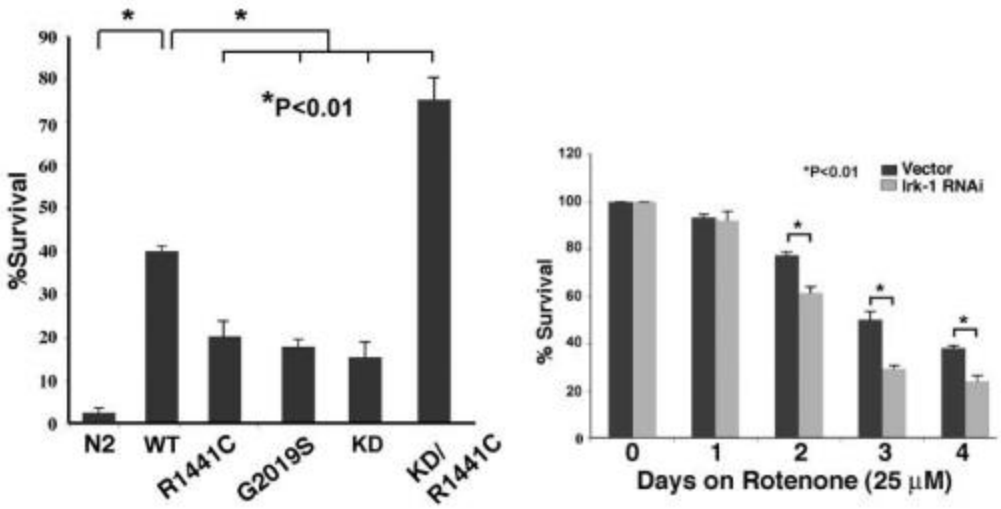


Fig 8(A) and 8(B): Survival rate of each strain of *LRRK2* expressing *C. elegans* shows protection conferred by WT *LRRK2* (Fig 8(A)). Removal of endogenous *lrk-1* in *C. elegans* makes them more susceptible to mitochondrial dysregulation and oxidative stress on rotenone (Fig 8(B)). Taken from Saha et al., 2009.

A final result of significance is that when a WT *LRRK2* and G2019S *LRRK2* strains of *C. elegans* were crossed with lines expressing DAT::GFP in worms that were age-synchronized in the larval stage and then followed into adulthood, the dopaminergic signal showed a rapid decrease in the *LRRK2* lines compared to the control DAT::GFP strain (Saha et al., 2009). But the G2019S *LRRK2* DAT::GFP showed a significantly greater decrease in the signaling- 80% less than the control line by day 2 of adult life. Figure 9 graphs the reduced DA levels in *LRRK2* strains by day 2 compared to control. By day 5, all strains showed reduced signaling with no significant difference between any

of the strains (Saha et al., 2009). These results corroborate the previously mentioned neurodegeneration observed by Yao et al., (2010).

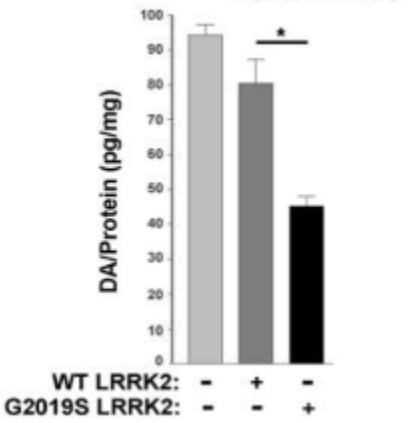


Fig 9: DA levels in WT vs G2019S strain. While DA levels are reduced in both *LRRK2* lines, levels are significantly reduced in the G2019S line at day 2 of adult life (*$p<0.05$). Taken from Saha et al., 2009.

With the role between *LRRK2* and mitochondrial stress protection shown through previous studies, more recent studies have focused on outlining the actual pathway or mechanism of *LRRK2* neuroprotection or the increased susceptibility to mitochondrial stress observed in G2019S *LRRK2* mutation. A 2018 study conducted by Long et al., hypothesized that G2019S lowers resistance to stress by inhibiting nuclear translocation of DAF-16, a process mediated by *ftt-2* in *C. elegans*. DAF-16 is a transcriptional regulator of genes that responds to and neutralizes the effects of oxidative stress (McElwee et al., 2003). This transcriptional factor has to translocate to the nucleus from the cytoplasm- a crucial step that is regulated by a 14-3-3 protein. These proteins are a

family of highly conserved proteins which are found in higher than normal amounts in the cerebrospinal fluid of Creutzfeldt-Jakob disease (Takahashi et al., 1999). In humans, patients with PD show a marked reduction in 14-3-3- protein (Long et al., 2018). *ftt-2* is one of the *C. elegans* isoforms of the 14-3-3 protein. The researchers generated *C. elegans* strains that express the human wild type *LRRK2*, G2019S *LRRK2* and G2019S D1994A KD *LRRK2* and then investigated the response of these different strains to thermal or oxidative stress. First, they crossed the three strains with a DAF-16::GFP (TJ356) strain to generate 3 new strains- WT(*LRRK2*)-TJ356, G2019S-TJ356 and KD-TJ356 strains. They then calculate the number of worms exhibiting DAF-16 after heat stress or induced oxidative stress through juglone treatment (Fig 10 (B)). It was found that in WT-TJ356 and G2019S-TJ356, DAF-16 is localized to the cytoplasm but in the TJ356 control strains, DAF-16 was localized in the nucleus (Fig 10(A)). Interestingly, the KD-TJ356 strain was able to rescue inhibition of DAF-16 nuclear translocation due to stress due to the non-functioning kinase and showed localization to nucleus (Long et al., 2018). This might point to a role for the kinase domain in the localization of DAF-16 in the cytoplasm in *C. elegans.*

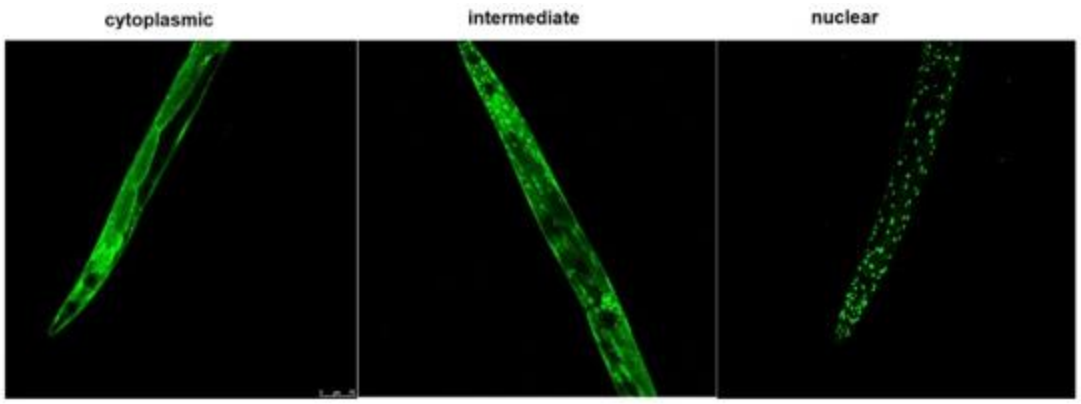


Fig 10 (A): Top panel- Depicts the three states of DAF-16 localization- cytoplasmic, both, or nuclear. Taken from Long et al., 2018

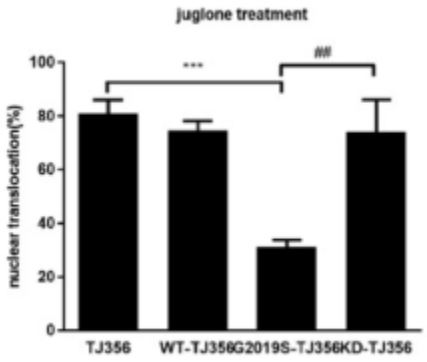


Fig 10 (B): Bottom panel-Graph representing the quantification of DAF-16 nuclear localization after mitochondrial stress treatment. Intermediate status was counted as nuclear localization. Only the GS2019 TJ356 strain showed significant cytoplasmic localization. ***$p<0.001$ & $^{\#\#\#}p<0.01$. Taken from Long et al., 2018.

Previous research had already shown that a 14-3-3 protein, called *ftt-2* in *C. elegans,* binds to DAF-16 and sequesters it in the cytoplasm (Li et al., 2007). The second result of significance in the present study, was that when all of these strains-

WT(*LRRK2*)-TJ356, G2019S-TJ356 and KD-TJ356 as well as the control TJ356 strain were fed an RNAi against *ftt-2*, DAF-16 was localized to the nucleus in WT-TJ356, KD-TJ356 and the controls strains but still localized in cytoplasm for the G2019S-TJ356 strain. The researchers suggest that G2019S inhibits *ftt-2* knockdown induced DAF-16 nuclear localization. While the exact mechanism of how G2019S does this is unknown it is kinase dependent since the KD strain was able to rescue nuclear localization (Long et al., 2018). Finally, the researchers also find that co-expressing human 14-3-3 fused with GFP into the three strains rescue neuronal death in all strains, but have the most effect on the G2019S strain (Long et al., 2018). This study was crucial in elucidating a pathway between one of the most common *LRRK2* mutations, G2019S, and a stress related transcriptional regulator DAF-16. Moreover, it provided a potential target for therapeutic intervention in the protective effects of 14-3-3 protein.

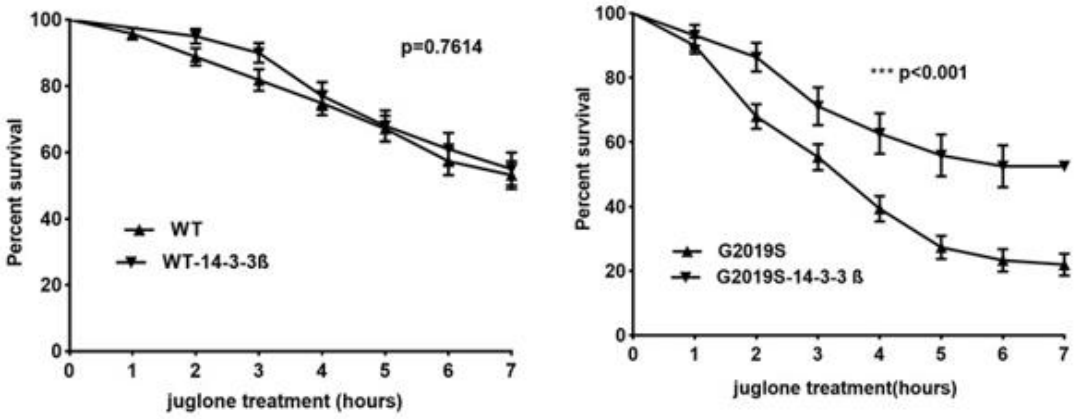


Fig 11: Survival rates of *C. elegans* strains after juglone treatment. (Left) While human 14-3-3 expression did not change survival rates in WT strain, it significantly increased survival in G2019S strain (***p<0.001). KD strain (not shown) also did not show significantly higher survival rates with 14-3-3 treatments. Taken from Long et al., 2018.

Overall, while the exact pathway of mitochondrial dysregulation induced by mutations in *LRRK2* in familial PD is still unknown, the role of DAF-16 as well as other regulators of stress response might be an important avenue of future research.

PINK1

PTEN induced kinase 1 (*PINK1*) is the second most common cause of autosomal recessive juvenile PD. *PINK1* encodes a 581 amino acid sequences for a serine-threonine kinase that is localized in the mitochondria (Shulman et al., 2011). But other studies have also shown portions of endogenous *PINK1* to be distributed in the cytoplasm as well (Sämann et al., 2009). In fact, the cytoplasmic kinase of *PINK1* has been shown to be essential in protection against mitochondrial stress (Haque et al., 2008). A common

substitution mutation in the *PINK1* gene, G309DM, leads to reduced striatal DAT binding in PD patients (Kessler et al., 2005).

In a study linking the function of *LRRK2* and *PINK1*, researchers found that loss of *C. elegans* homolog of *PINK1, pink-1*, results in oxidative stress and neurite defects but these cellular defects were reversed when *lrk-1*, the *C. elegans* homolog of *LRRK2*, was removed from the nematode (Sämann et al., 2009). Interestingly, the researchers of this present study used GFP marker to determine the localization of *pink1* in the *C. elegans* body and found a similar pattern of distribution as humans- both in mitochondria and in the cytoplasm. This supports research done in humans and can point to functions of *pink-1/PINK1* beyond the nervous system. To determine the effect of loss of *pink-1* on mitochondrial homeostasis, a null allele of the gene was introduced, tm1779, which resulted in complete loss of function. When these nematodes were exposed to paraquat induced toxicity, survival rates were significantly lower in the tm1779 strain as compared to wild type. Expression of transgenic *pink-1* in these mutants restored survival rates (Fig 12). This establishes a role for *pink-1* in neuroprotection against mitochondrial stress which has been further been confirmed in studies performed in *D. melanogaster* (Park et al., 2006). Additionally, in the tm1779 strain, the length of the mitochondrial cristae was reduced by 30% in neurons compared to wild type confirming a role for *pink-1* in maintaining mitochondrial integrity (Fig 13 B) (Sämann et al., 2009).

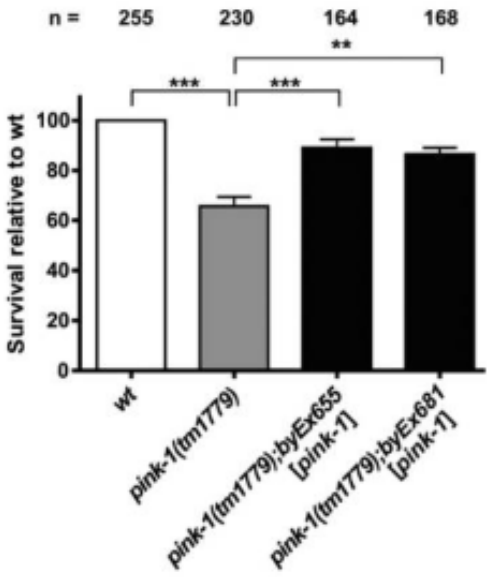


Fig 12: Graph depicting survival rates of strains normalized to WT. The tm1779 strain had lower survival rates when compared to all other strains. **p<0.001 and ***p<0.0007. Taken from Sämann et al., 2009.

To establish a connection between *LRRK2* and *PINK-1*, the *C. elegans* homolog, *lrk-1* was separated into two strains - *lrk-1*(tm1898) and *lrk-1*(km41) both strains containing deletion mutations in the kinase domain. However unlike previous researchers, this study found no significant difference in survival rates for these *lrk-1* mutated strains. This could be explained by results seen in the kinase dead strains discussed in the *LRRK2* section. However, when the two mutated *lrk-1* strains were combined with the *pink-1* mutant and then subject to mitochondrial stress, the resultant progeny had a significantly higher survival rate than the *pink-1* strain alone (Fig 13 A). Additionally, when mitochondrial cristae defects were studied, the *lrk-1* mutated strains

were identical to wild type. But surprisingly, the double mutated strain also looked identical to wild type thus reversing the cristae defect seen in the *pink-1* mutated strain alone (Fig 13 B) (Sämann et al., 2009).

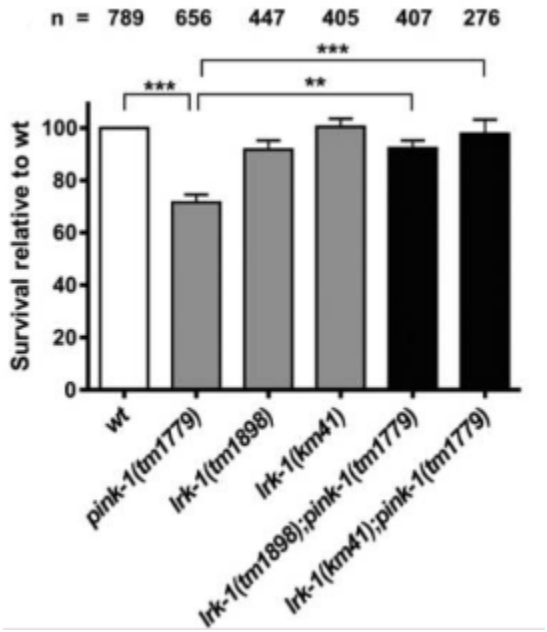


Fig 13 (A): Graph depicting lower survival rates of the pink-1 mutants. Double mutated *pink-1, lrk-1* strain has a significantly higher survival rate comparable to wild type. **p<0.001 & ***p<0.0007. Taken from Sämann et al., 2009.

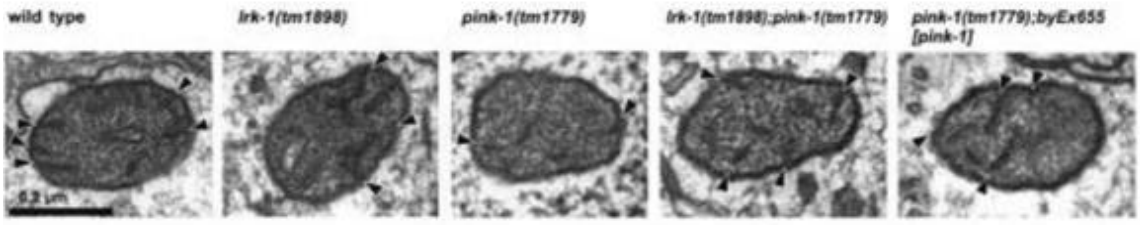


Fig 13 (B): Cross section of mitochondria in *C. elegans* neurons. As shown by the arrows, the *pink-1* mutated strain has significant reduction in cristae length. Taken from (Sämann et al. 2009).

Consistent with the findings of this study, recent studies with *PINK-1* knock-out mice models have shown severe defects in many ETC components and enzymes such as complex I, II-IV along with reduced aconitase activity and impaired cellular respiration (Gautier et al., 2008). Collectively, these studies indicate a common pathway and antagonist function between *LRRK2* and *PINK1* in maintaining mitochondrial integrity and regulation.

Parkin

Loss of function mutation of the *Parkin* gene is the most common cause of autosomal recessive juvenile PD. This form of PD was first observed in 1998 in Japanese families and was shown to have chromosome 6 linkage. The *Parkin* gene encodes a 465 amino acid protein, similar to E3 ubiquitin ligases, which targets cytoplasmic proteins for degradation in the proteasome. *Parkin* associated disease has a phenotype that is similar to PD in its motor symptoms and response to L-DOPA. However, this latter disease diverges from PD in that the age of onset is much younger and with symptoms that are atypical of PD such as dystonia, reflex changes, diurnal symptomatic fluctuations and in pathology there is a lack of presence of Lewy Bodies (Shulman et al., 2011). *Parkin* was also shown to form an E3 ligase complex with *DJ-1* and *PINK1* (Xiong et al., 2009). *Parkin* knockout mice show increased extracellular DA, which as mentioned before may act as a neurotoxin, due to reduced DAT availability (Jiang et al., 2004), impaired synaptic plasticity and DA neurodegeneration with loss of excitatory synapse (Kitada et al., 2009, Helton et al., 2008).

While the pathway between some of these genes and gene products like *PINK-1* and *LRRK2* are still being outlined, there is substantial evidence of interaction between *Parkin* and *PINK-1*. Since *PINK-1* is a kinase, one of its phosphorylation targets is *Parkin* and this is phosphorylation step essential to the ubiquitin ligase transfer activity (Iguchi et al. 2013). This role of *Parkin* is especially important since it initiates a series of steps responsible for autophagy ("mitophagy") of the mitochondria that are damaged through oxidative stress (Vivez-Bauza et al. 2010). In *D. melanogaster* model of PD, *PINK-1* mutants shared many of the same phenotypic characteristics as *Parkin* mutants but more importantly, transgenic expression of *Parkin* alleviated all loss-of-function symptoms of *PINK-1* but expression of *PINK-1* did not do the same for *Parkin*. The researchers of that study suggest that this means *Parkin* acts downstream of *PINK-1* (Park et al., 2006). The plethora of research in the field of PD done on *PINK-1* & *Parkin* interaction suggest that perhaps PD pathophysiology occurs from ineffective autophagy of mitochondria under stress conditions due to mutations in *PINK-1* & *Parkin* which ultimately may lead to neurodegeneration. In a previously mentioned study under the *LRRK2* section, researchers found that while *LRRK2* interacts with *Parkin* but not α-synuclein in co-immunoprecipitation studies (Smith et al. 2005). However, unlike the hypothesized antagonistic function between *PINK-1* and *LRRK2*, co-expression of *Parkin* in *LRRK2* mutant nematode strains did not protect against neurodegeneration suggesting that these two gene products might not have antagonistic functions (Smith et al., 2005).

A recent 2017 study discovered a novel pathway in *C. elegans* for the extrusion of misfolded protein and dying mitochondria in a pathway separate from traditional

pathways such as use of chaperones, protein degradation, and autophagy/mitophagy (Melentijevic et al., 2017). Adult *C. elegans* were found to extrude large (4 μm) membrane bound vesicles called "exophers" that contain misfolded proteins and organelles. Researchers first noticed these vesicles when fluorescent signals started appearing from outside cells in what appeared to be large vesicles. The fascinating feature of these vesicles, in relation to PD, is that they're produced by multiple neuronal types including dopaminergic PDE and CEP neurons. Figure 14 shows the extrusion of one of these "exophers" from the soma of an ALM neuron. Researchers observed that the size of the exopher gradually increased over an hour but fluorescence intensity also increased suggesting that material was being delivered into the vesicle even after extrusion (Melentijevic et al., 2017). These vesicles did not stain for nuclear material and were not found to be a result of cell division (Melentijevic et al., 2017).

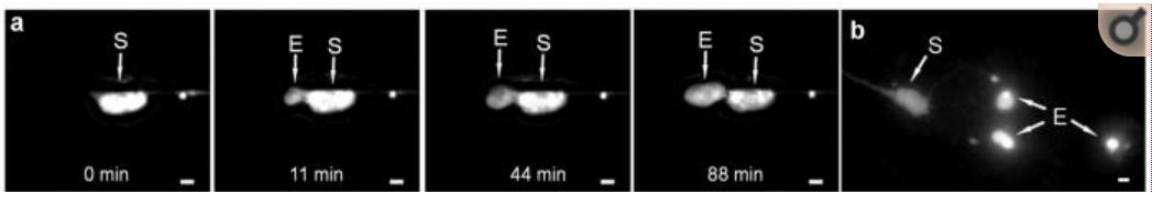


Fig 14: Image of an exopher (E) being extruded out of some of an ALM neuron (S) and containing intense fluorescent staining visualized with mCherry staining for protein visualization. Taken from Melentijevic et al., 2017.

Rather than being neurotoxic, these exophers seem protective in their function since the functionality of neurons that generate exophers is retained over neurons that did

not generate exophers when both these types of neurons underwent proteotoxic stress conditions (Melentijevic et al., 2017). Furthermore, due to their large size researchers hypothesized that these vesicles may even contain damaged/apoptotic organelles like mitochondria and lysosomes. Fig 15 (A) shows the image of a mitochondria inside a budding exopher, which tends to form a rind around the somatic periphery and often have its own exopher. Interesting, when genes important to mitochondrial health such as *PINK-1/pink-1, ubl-5* (mitochondrial unfolded protein response), *BNIP3/dct-1* (mitophagy), and *Parkin* homolog, *pdr-1*, were genetically manipulated through RNAi knockdown, exopher extrusion increased in touch sensitive ALMR neurons (Fig 15 B). In addition, researchers found that juglone treated nematode strains had significantly higher numbers of mitochondria including exophers than matched controls. This confirmed that stressed/damaged mitochondria are preferentially extruded in exophers (Melentijevic et al., 2017).

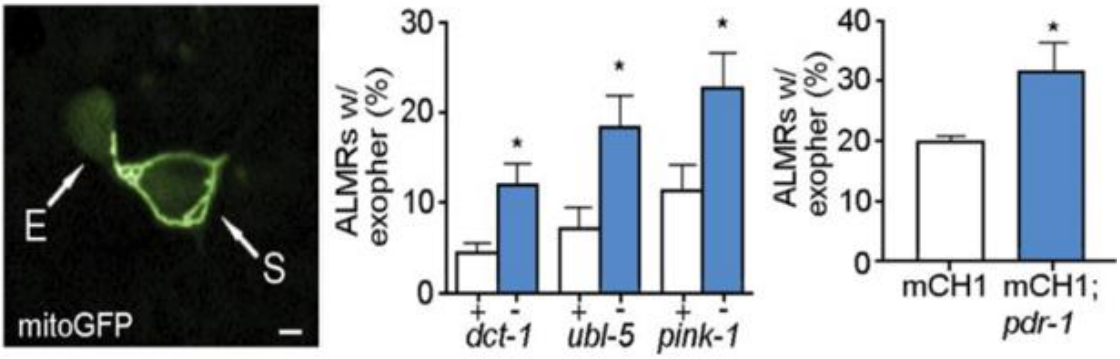


Fig 15 (A): (left) Image of a mitochondria being extruded into a budding exopher (E) from the soma of a neuron (S) visualized using mitoGFP. Taken from Melentijevic et al., 2017.

Fig 15 (B): (Right-graphs) RNAi knockdown of genes essential to mitochondrial integrity- *pink-1, ubl-1, dct-1* and *pdr-1* shown in blue bars compared to control empty vectors (white). Taken from Melentijevic et al., 2017.

Overall, this study was able to discover a novel pathway by which damaged mitochondria are expelled out of dopaminergic neurons and the role the upregulation of this pathway when integral mitochondrial house-keeping genes such as *park-1* and *pdr-1* are mutated. Studies in mice model have shown that retinal cells often extrude damaged mitochondria into neighboring astrocytes for degradation (Davis et al., 2014). With the confirmation of mitochondrial expulsion under stress in both nematode and a mammalian model, the role of mitochondrial expulsion in quality control of damaged neurons may be an avenue of future interest for researchers studying neurodegenerative or even prion related diseases.

Toxin Based Models of PD

Since environmental/ sporadic factors are the major cause of PD, it is important to understand how some environmental toxins and heavy metals may play a part in mitochondrial dysregulation and oxidative stress that leads to damage to dopaminergic neurons in PD. This is especially important to understand since these toxins are often used to mimic the pathogenesis of PD in laboratory research and to make any meaningful conclusion from these studies one has to understand gene-environment interactions.

MPTP/ 6-OHDA

MPTP toxicity was first discovered in substance users in California when it was found to be an additive in synthetic heroin (Langston et al., 1983). MPTP is lipophilic, can cross the BBB, and under the action of MAO-B in the brain is converted into MPP+. As mentioned earlier, MPP+ is a neurotoxin and acts on the mitochondria by inhibiting complex I, decrease ATP production, decrease mtDNA, and reduce the autophagy capabilities of the mitochondria (Abou-Saleim et al., 2006). Treating *C. elegans* with MPP+ has been shown to lead to marked degeneration of DA neurons along with motor deficits like reduced mobility. However, administering DA receptor agonists improved mobility in wild type nematodes (Braungart et al., 2004).

Similarly, 6-OHDA, an oxidative product of DA, is another known neurotoxin in mammals and in both mammals and *C. elegans* has shown to decrease the number of dopaminergic neurons (Nass et al., 2002). Interestingly, 6-OHDA is found in the urine of patients with PD, which might account for its production *in vivo* in humans as part of the pathology of PD (Jellinger et al., 1995). Both these neurotoxins, MPP+ and 6-OHDA, selectively accumulate in neurons through DAT increasing the oxidative stress load/ROS and thereby leading to mitochondrial dysfunction (Javitch et al.,1985). To study the toxicity of 6-OHDA on *C. elegans* dopaminergic neurons, researchers exposed nematodes to a liquid suspension of 6-OHDA for an hour and then transferred the worms out of the toxic media and into regular ones. On imaging within 2 hours of exposure, CEP and ADE neuron processes were found to be rounded (Fig 16 A-B), and within 72 hours of exposure, complete loss of GFP expression is seen (Fig 16 C) (Nass et al., 2002).

However, when co-administered with a DAT inhibitor, imipramine, dramatically improved neurons comparable to untreated worms were seen (Fig 16 D). These latter results could be replicated with the administration of D-amphetamine, a psychoactive drug, as well (Nass et al., 2002).

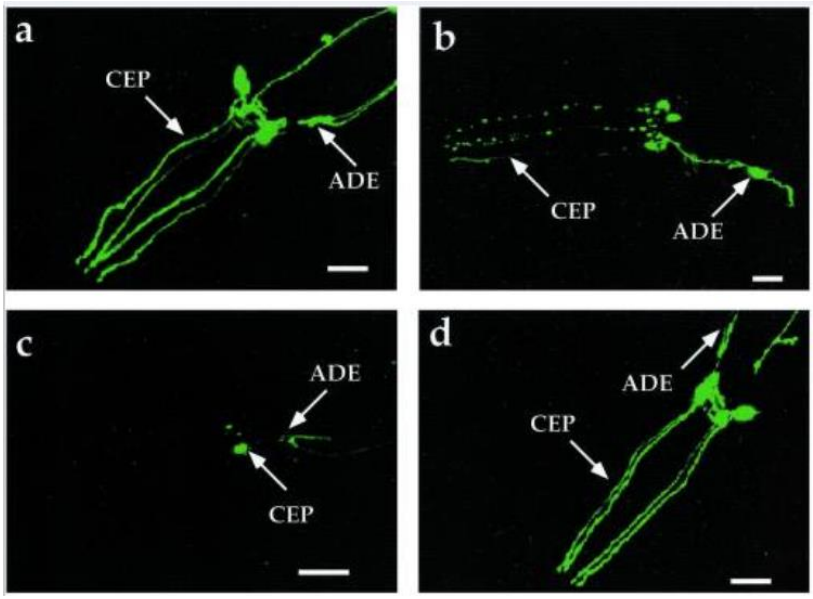


Fig 16 (A-C) depicts the gradual loss of DA signaling from untreated to 72 hours after treatment and

Fig 16 (D) depicts results after co-administration of a potentially therapeutic agent, imprimanie. Taken from Nass et al., 2002.

DISCUSSION

This review aimed to recount some of the current and past research in the field of Parkinson's disease and specifically with the use of the worm model *C. elegans.* Specifically, this review focused on the genetic models of *C. elegans* used in PD research to elucidate if a similar pathway operated in the human version of the disease. Mutations in *LRRK2*, whose gene product contains both a GTPase and a kinase domain, such as the G2019S mutation which uncouples mitochondrial oxidative phosphorylation (Mortiboys et al., 2010) have been shown to be an extremely significant factor in mitochondrial dysfunction. Expression of this gene in *C. elegans* leads to age-dependent neurodegeneration (Yao et al., 2010) as well as a loss of DA signaling as quantified through GFP staining (Saha et al. 2009). The nematode's own homolog of *LRRK2, lrk-1,* has been linked to synaptic vesicle polarization. When a knock-out of this gene, *lrk-1,* was performed lower survival rates post-mitochondrial stress treatment was observed in the worms (Saha et al. 2009). However, when the kinase domain is deleted (Saha et al. 2009) or a GTPase binding mutation is introduced (Yao et al. 2010), the neurodegeneration was reversed (Yao et al. 2010) and survival rates of worms were similar to WT in mitochondrial stress conditions (Saha et al. 2009). More importantly, in the otherwise destructive strain R1441C, which causes neurodegeneration in worms (Yao et al. 2010) as well as lower survival rates (Saha et al. 2009), introducing a mutation in the kinase domain reversed these results. Taken together, these results outline an important function for both the GTPase as well as the Kinase domain of *LRRK2* in the

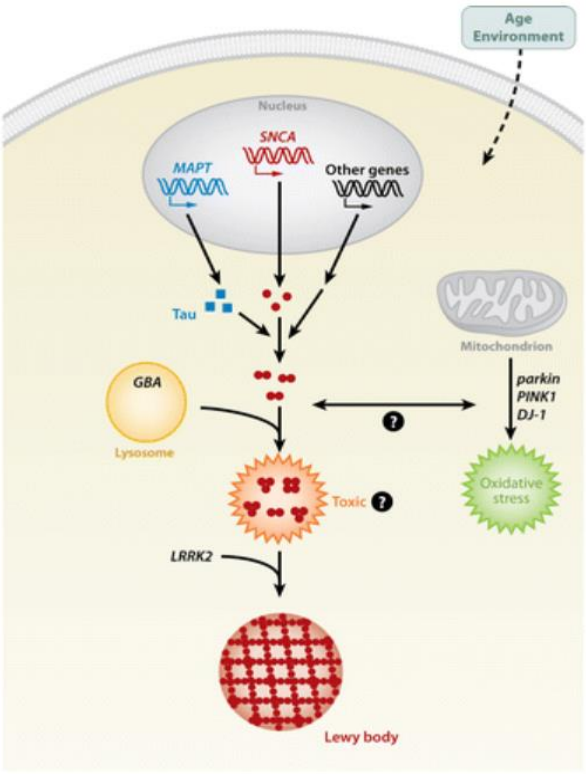
neurodegeneration witnessed in *LRRK2* mutant strains. These studies also confirm the theory that *LRRK2* is neuroprotective and that mutation in this gene/gene product might be the cause of neurodegeneration since re-introducing the worm's own *lrk-1* or ectopic expression of human *LRRK2* reverses the neurodegeneration seen in mutants (Saha et al. 2009). The recent study conducted by Long et al. (2018) was crucial in connecting the gaps in the theory of how G2019S mutations cause mitochondrial stress and ultimately neurodegeneration. Patients with PD were observed to have low levels of 14-3-3 protein, which regulates the localization of DAF-16, a transcriptional regulator of genes that responds to and neutralizes the effects of oxidative stress, into the nucleus. During stress conditions, 14-3-3 protein is inhibited to allow for the translocation of DAF-16 to the nucleus. Researchers found that when *LRRK2* and its mutated version G2019S are introduced in the worm, localization of DAF-16 is in the cytoplasm but in the kinase dead strain localization is in the nucleus like the control strains. This indicated that the kinase domain was important in the translocation of the transcription regulator. Furthermore, when researchers eliminated *ftt-2*, the worm's own version of the 14-3-3 protein, then nuclear localization was seen in all the strains except the G2019S strain where DAF-16 was still localized to the cytoplasm. This suggested that G2019S blocked 14-3-3 knockdown induced translocation of DAF-16 in a kinase dependent manner (Long et al. 2018). The researchers hypothesize that perhaps G2019S phosphorylates DAF-16 leading to it being sequestered in the cytoplasm. While the exact mechanism is unknown, this research confirms a new potential therapeutic target in the form of the 14-3-3 protein.

Interestingly, while *lrk-1* confers protection to the worms from oxidative stress, when *pink-1*, a homolog of *PINK-1*, is mutated in *C. elegans*, strains exhibits lowered tolerance to parquat (a mitochondrial stress agent) and shortening of mitochondria cristae is observed (Sämann et al., 2009). But when *lrk-1* is also mutated in these worms, a reversal of the previously observed changes were observed. This suggested that while each gene individually confers neuroprotection, *lrk-1* is unable to compensate for the loss of *pink-1*, which leads to mitochondrial defects and lower survival rates. Researchers suggest that *lrk-1* could be a downstream factor that is negatively regulated by *pink-1* (Sämann et al., 2009). So, mutations or loss-of-function of *pink-1* lead to an increase in *lrk-1* expression but when this *lrk-1* is also deleted then basal levels are restored and suppress the *pink-1* deletion phenotype (shortened mitochondria cristae). However, this explanation does not corroborate with the neuroprotection conferred by *lrk-1* seen in previous studies (Saha et al. 2009). If *pink-1* mutation leads to increased *lrk-1* activity than increased neuroprotection and hence higher survival rates of worms should be seen. However, the researchers also hypothesize that *lrk-1* is downstream of *pink-1* as evidenced by the observation that while *lrk-1* mutation can rescue the *pink-1* mutation, the reverse is not true (Sämann et al., 2009). Since mitochondrial defects are only observed in *pink-1* but not *lrk-1* mutants, perhaps over-expression of *lrk-1* cannot compensate for the lack of pink-1. Further research is needed to confirm if *pink-1* is the single most important gene for mitochondrial health amongst the mitochondrial maintenance genes. While expression of *lrk-1* was not sufficient to alleviate the loss of function symptoms of *pink-1* (but mutation of *lrk-1* was), expression of *Parkin*, another

mitochondrial maintenance gene implicated in PD, was sufficient to reverse all loss of function symptoms seen in *D. melanogaster* model of PD. From the studies done in *C. elegans* and *D. melanogaster* discussed here, it could be hypothesized that perhaps both *Parkin* and *LRRK2* act downstream of *PINK-1* and that while *PINK-1/pink-1* negatively regulates *LRRK2/lrk-1*, it positively regulates *Parkin* expression. While such a study has not been performed in *C. elegans*, a knockout study using RNAi or mutated alleles that knocks-down each of the three discussed genes, as well as *DJ-1* (not discussed in this review), and then studying the symptoms of the mutations along with effects of a double knock-down of a second gene could be critical in understanding the role of each gene with respect to each other.

Finally, while the presence of exophers was only elucidated recently, the presence of damaged mitochondria in these extruded vesicles is particularly interesting (Melentijevic et al., 2017). Since *PINK-1* and *Parkin* have been shown to be integral to maintain mitochondrial health, the increased presence of exophers in strains in which *PINK-1* and *Parkin* homolog, *pdr-1*, are knocked-down add a new element of consideration (Melentijevic et al., 2017). This is because it adds another pathway that neurons use to extrude stress damaged mitochondria, mitophagy (mitochondrial autophagy), that has not been previously studied. The presence of such an alternative pathway in *D. melanogaster* or even a higher vertebrate model could be crucial in our understanding of mitochondrial maintenance by these genes and hence the dysregulation caused by mutations of these genes.

While the central culprit for PD is still thought to be α-synuclein aggregation leading to Lewy Body formation, other pathways and genes interact with α-synuclein to contribute towards what is largely observed in the pathology of PD. Fig 17 illustrates the theory that some scientists and researchers prescribe to. According to Shulman et al., (2011) *SNCA* gene expression and accumulation of α-synuclein is the trigger for the cascade that eventually leads to oxidative stress and finally neurodegeneration. The researchers suggest that other susceptibility genes that are responsible for other pathways then interact with the α-synuclein triggered chain of events. For example- Glucocerebrosidase (*GBA)* is implicated in the lysosomal degradation machinery and Microtubule-associated protein tau (*MAPT)* is related to tau, largely associated with Alzheimer's Diseases, thus connecting PD to the larger field of ND research. The genes talked about in this review paper- *LRRK2, Parkin*, and *PINK-1* might thus work alongside these other pathways in dysregulating normal mitochondrial function and contributes to the production of ROS and ultimately oxidative stress. Environmental agents can add to the oxidative load in this pathway (Shulman et al., 2011). Fig 17 illustrates a simplistic depiction of the theory laid out above however the specific ways in which these pathways communicate with each other is still unknown. The eventual outcome is the formation of Lewy Bodies but the inability of pathways to self-regulate, might help us understand why neuronal death eventually occurs. Simplistic animal models like *C. elegans* can be of significant value in elucidating an all-encompassing theory.



Shulman JM, et al. 2011.
Annu. Rev. Pathol. Mech. Dis. 6:193–222

Fig 17: Theory of progression of PD starting with α-synuclein aggregation and eventually interacting with *GBA, MAPT, LRRK2* and other genes of susceptibility. Taken from Shulman et al 2011.

REFERENCES

Abou-Sleiman PM, Muqit MM, Wood NW. (2006). Expanding insights of mitochondrial dysfunction in Parkinson's disease. *Natural Reviews Neuroscience*; 7:207–219.

Arai R., Karasawa N., Geffard M., Nagatsu I. (1995). L-DOPA is converted to dopamine in serotonergic fibers of the striatum of the rat: a double-labeling immunofluorescence study. *Neuroscience*. Lett.195, 195–198. 10.1016/0304-3940(95)11817-G

Arai R., Karasawa N., Geffard M., Nagatsu T., Nagatsu I. (1994). Immunohistochemical evidence that central serotonin neurons produce dopamine from exogenous L-DOPA in the rat, with reference to the involvement of aromatic L-amino acid decarboxylase. *Brain Research.* 667, 295–299. 10.1016/0006-8993(94)91511-3

Bernheimer, H., Birkmayer, W., Hornykiewicz, O., Jellinger, K., & Seitelberger, F. (1973). Brain dopamine and the syndromes of Parkinson and Huntington. Clinical, morphological and neurochemical correlations. *Journal of the Neurological Sciences*, *20*(4), 415–455.

Caldwell, G. A., & Caldwell, K. A. (2008). Traversing a wormhole to combat Parkinson's disease. *Disease models & mechanisms*, *1*(1), 32–36. doi:10.1242/dmm.000257

Beaulieu JM, Gainetdinov RR. (2011).The physiology, signaling, and pharmacology of dopamine receptors. *Pharmacological Reviews*. 2011;63:182–217

Bonifati, V., et al. (2002). Mutations in the DJ-1 gene associated with autosomal recessive early-onset Parkinsonism. *Science.* doi:10.1126/science.1077209

Biskup, S., Moore, D. J., Celsi, F., Higashi, S., West, A. B., Andrabi, S. A., … Dawson, V. L. (2006). Localization of LRRK2 to membranous and vesicular structures in mammalian brain. *Annals of Neurology*, *60*(5), 557–569.

Braungart E., Gerlach M., Riederer P., Baumeister R., Hoener M. C. (2004). Caenorhabditis elegans MPP+ model of Parkinson's disease for high-throughput drug screenings. *Neurodegeneration. Dis.* 1, 175–183. 10.1159/000080983

Chakraborty, S., Bornhorst, J., Nguyen, T. T., & Aschner, M. (2013). Oxidative stress mechanisms underlying Parkinson's disease-associated neurodegeneration in C.

elegans. *International journal of molecular sciences*, *14*(11), 23103–23128. doi:10.3390/ijms141123103

Chen, X., Barclay, J. W., Burgoyne, R. D., & Morgan, A. (2015). Using C. elegans to discover therapeutic compounds for ageing-associated neurodegenerative diseases. *Chemistry Central journal*, *9*, 65. doi:10.1186/s13065-015-0143-y

Chege, P. M., & McColl, G. (2014). Caenorhabditis elegans: a model to investigate oxidative stress and metal dyshomeostasis in Parkinson's disease. *Frontiers in aging neuroscience*, *6*, 89. doi:10.3389/fnagi.2014.00089

Consortium C. E. S. (1998). Genome sequence of the nematode *C. elegans*: a platform for investigating biology. Science 282, 2012–2018 10.1126/science.282.5396.2012

Corsi A.K., Wightman B., and Chalfie M. (2015). A Transparent window into biology primer on Caenorhabditis elegans, WormBook, ed. The C. elegans Research Community, WormBook. doi/10.1895/wormbook.1.177.1

Davis, C. H., Kim, K. Y., Bushong, E. A., Mills, E. A., Boassa, D., Shih, T., … Marsh-Armstrong, N. (2014). Transcellular degradation of axonal mitochondria. *Proceedings of the National Academy of Sciences of the United States of America*, *111*(26), 9633–9638. doi:10.1073/pnas.1404651111

Dawson, T. M., & Dawson, V. L. (2003). Rare genetic mutations shed light on the pathogenesis of Parkinson disease. *The Journal of clinical investigation*, *111*(2), 145–151. doi:10.1172/JCI17575

Dawson VL. (2006). Localization of LRRK2 to membranous and vesicular structures in mammalian brain. *Annals of Neurology*. 60:557–569

Elsworth, J. D., & Roth, R. H. (1997). Dopamine synthesis, uptake, metabolism, and receptors: relevance to gene therapy of Parkinson’s disease. *Experimental Neurology*, *144*(1), 4–9.

Exner N, Treske B, Paquet D, Holmstrom K, Schiesling C, Gispert S, Carballo-Carbajal I, Berg D, Hoepken HH, Gasser T, Kruger R, Winklhofer KF, Vogel F, Reichert AS, Auburger G, Kahle PJ, Schmid B, Haass C. Loss-of-function of human PINK1 results in mitochondrial pathology and can be rescued by parkin. *Journal of Neuroscience*. 2007;27:12413–12418.

Fahn S., Libsch L. R., Cutler R. W. Monoamines in the human neostriatum: topographic distribution in normals and in Parkinson's disease and their role in akinesia, rigidity,

chorea, and tremor. *Journal of the Neurological Sciences*. 1971;14(4):427–455. doi: 10.1016/0022-510x(71)90178-x

Ford C. P. (2014). The role of D2-autoreceptors in regulating dopamine neuron activity and transmission. *Neuroscience, 282,* 13–22. doi:10.1016/j.neuroscience.2014.01.025

Gandhi PN, Chen SG, Wilson-Delfosse AL. (2009). Leucine-rich repeat kinase 2 (LRRK2): a key player in the pathogenesis of Parkinson's disease. *Journal of Neuroscience*. Res, 87:1283–1295.

Gasnier, B. (2000). The loading of neurotransmitters into synaptic vesicles. *Biochimie, 82*(4), 327–337.

Gautier, C. A., Kitada, T., & Shen, J. (2008). Loss of PINK1 causes mitochondrial functional defects and increased sensitivity to oxidative stress. *Proceedings of the National Academy of Sciences of the United States of America, 105*(32), 11364–11369.

Gegg M. E., Burke D., Heales S. J., Cooper J. M., Hardy J., Wood N. W., et al. . (2012). Glucocerebrosidase deficiency in substantia nigra of Parkinson disease brains. *Annals of Neurol.* 72, 455–463. 10.1002/ana.23614

Greggio E, Jain S, Kingsbury A, Bandopadhyay R, Lewis P, Kaganovich A, van der Brug MP, Beilina A, Blackinton J, Thomas KJ, Ahmad R, Miller DW, Kesavapany S, Singleton A, Lees A, Harvey RJ, Harvey K, Cookson MR.(2006).Kinase activity is required for the toxic effects of mutant LRRK2/dardarin. *Neurobiology of Disease*. 23:329–341.

Grover S., Somaiya M., Kumar S., Avasthi A. (2015). Psychiatric aspects of Parkinson's disease. *Journal of Neuroscience.* Rural Pract. 6, 65–76. 10.4103/0976-3147.143197

Guo M. (2012). Drosophila as a model to study mitochondrial dysfunction in Parkinson's disease. *Cold Spring Harbor Perspectives in Medicine* .2, 1–18. 10.1101/cshperspect.a009944

Guo L, Wang W, Chen SG. Leucine-rich repeat kinase 2: Relevance to Parkinson's disease. *The International Journal of Biochemistry & Cell Biology*. Cell Biol. 2006; 38:1469–1475.

Haque M. E., Thomas K. J., D'Souza C., Callaghan S., Kitada T., Slack R. S., Fraser P., Cookson M. R., Tandon A., Park D. S. (2008). Cytoplasmic Pink1 activity protects neurons from dopaminergic neurotoxin MPTP. *Proceedings of National Academy of Sciences of the U. S. A.* 105, 1716– 1721

Harrington, A. J., Hamamichi, S., Caldwell, G. A., & Caldwell, K. A. (2010). C. elegans as a model organism to investigate molecular pathways involved with Parkinson's disease. *Developmental Dynamics*, NA-NA. https://doi.org/10.1002/dvdy.22231

Helton T.D., Otsuka T., Lee M.C., Mu Y., Ehlers M.D. (2008). Pruning and loss of excitatory synapses by the parkin ubiquitin ligase. *Proceedings of National Academy of Sciences of the U. S. A*. Acad. Sci. USA. 105:19492–19497.'

Huot, P., Fox, S. H., & Brotchie, J. M. (2015). Monoamine reuptake inhibitors in Parkinson's disease. *Parkinson's disease*, *2015*, 609428. doi:10.1155/2015/609428

Iguchi M., Kujuro Y., Okatsu K., Koyano F., Kosako H., Kimura M., Suzuki N., Uchiyama S., Tanaka K., Matsuda N. (2013).Parkin catalyzed ubiquitin-ester transfer is triggered by PINK1-dependent phosphorylation. *The Journal of Biological Chemistry*. 2013; 288:22019–22032

Jagmag, S. A., Tripathi, N., Shukla, S. D., Maiti, S., & Khurana, S. (2016). Evaluation of Models of Parkinson's disease. *Frontiers in neuroscience*, *9*, 503. doi:10.3389/fnins.2015.00503

Javitch, J. A., D'Amato, R. J., Strittmatter, S. M., & Snyder, S. H. (1985). Parkinsonism-inducing neurotoxin, N-methyl-4-phenyl-1,2,3,6 -tetrahydropyridine: uptake of the metabolite N-methyl-4-phenylpyridine by dopamine neurons explains selective toxicity. *Proceedings of the National Academy of Sciences of the United States of America*, *82*(7), 2173–2177.

Jellinger K, Linert L, Kienzl E, Herlinger E, Youdim M B. J Neural Transm. (1995). Chemical evidence for 6-hydroxydopamine to be an endogenous toxic factor in the pathogenesis of Parkinson's disease. *Journal of Neural Transmission.* Suppl., 46:297–314.

Jiang H., Jiang Q., Feng J. (2004). Parkin increases dopamine uptake by enhancing the cell surface expression of dopamine transporter. *The Journal of Biological Chemistry*.. *2*79:54380–54386.

Kessler K.R., Hamscho N., Morales B., Menzel C., Barrero F., Vives F., Gispert S., Auburger G. (2005). Dopaminergic function in a family with the PARK6 form of autosomal recessive Parkinson's syndrome. *Journal of Neural Transmission.* 112:1345–1353

Kokhan V. S., Afanasyeva M. A., Van'kin G. I. (2012). alpha-Synuclein knockout mice have cognitive impairments. *Behavioral Brain Research*. 231, 226–230 10.1016/j.bbr.2012.03.026 75–980

Kitada T., Pisani A., Karouani M., Haburcak M., Martella G., Tscherter A., Platania P., Wu B., Pothos E.N., Shen J. (2009). Impaired dopamine release and synaptic plasticity in the striatum of parkin−/− mice. *Journal of Neurochemistry.* 110:613–621.

Krige D, Carroll MT, Cooper JM, Marsden CD, Schapira AH The Royal Kings and Queens Parkinson Disease Research Group. Platelet mitochondrial function in Parkinson's disease. *Annals of Neurology*. 1992;32:782–788

Lambert, A. J., & Brand, M. D. (2009). Reactive Oxygen Species Production by Mitochondria. In J. A. Stuart (Ed.), *Mitochondrial DNA: Methods and Protocols* (pp. 165–181). Totowa, NJ: Humana Press.

Langston JW, Ballard P, Tetrud JW, & Irwin I (1983) Chronic Parkinsonism in humans due to a product of meperidine-analog synthesis. *Science*. 219, 979–980.

Lees, AJ, Hardy, J, Revesz, T. (2009). Parkinson's disease. *Lancet* 373: 2055– 2066.

Li, J., Tewari, M., Vidal, M., & Lee, S. S. (2007). The 14-3-3 protein FTT-2 regulates DAF-16 in Caenorhabditis elegans. *Developmental Biology*, *301*(1), 82–91.

Long, S., Guo, W., Hu, S., Su, F., Zeng, Y., Zeng, J., … Pei, Z. (2018). G2019S LRRK2 Increases Stress Susceptibility Through Inhibition of DAF-16 Nuclear Translocation in a 14-3-3 Associated-Manner in *Caenorhabditis elegans*. *Frontiers in neuroscience*, *12*, 782. doi:10.3389/fnins.2018.00782

Maulik, M., Mitra, S., Bult-Ito, A., Taylor, B. E., & Vayndorf, E. M. (2017). Behavioral Phenotyping and Pathological Indicators of Parkinson's Disease in *C. elegans* Models. *Frontiers in genetics*, *8*, 77. doi:10.3389/fgene.2017.00077

McElwee J., Bubb K., Thomas J. H. (2003). Transcriptional outputs of the *Caenorhabditis elegans* forkhead protein DAF-16. *Aging Cell* 2 111–121. 10.1046/j.1474-9728.2003.00043.x

Melentijevic, I., Toth, M. L., Arnold, M. L., Guasp, R. J., Harinath, G., Nguyen, K. C., … Driscoll, M. (2017). C. elegans neurons jettison protein aggregates and mitochondria under neurotoxic stress. *Nature*, *542*(7641), 367–371. doi:10.1038/nature21362

Nass, R., Merchant, K. M., & Ryan, T. (2008). Caenorhabditis elegans in Parkinson's Disease Drug Discovery: Addressing an Unmet Medical Need. *Molecular Interventions*, *8*(6), 284–293. https://doi.org/10.1124/mi.8.6.6

Nass, R., Hall, D.H., Miller, D.M., and Blakely, R.D. (2002). Neurotoxin-induced degeneration of dopamine neurons in *Caenorhabditis elegans. Proceedings of the National Academy of Sciences of the United States of America. Sci.* 99, 3264–3269

Nass, R. and Nichols, C.D. (2007). Invertebrates as powerful genetic models for human neurodegenerative disease. *Handbook of Contemporary Neuropharmacology* (Sibley, D.R., Hanin, I., Kuhar, M., and Skolnick, P. eds) pp 567–588

Navailles, S., Bioulac, B., Gross, C., & De Deurwaerdère, P. (2011). Chronic L-DOPA therapy alters central serotonergic function and L-DOPA-induced dopamine release in a region-dependent manner in a rat model of Parkinson's disease. *Neurobiology of Disease*, *41*(2), 585–590.

Nirenberg, M. J., Vaughan, R. A., Uhl, G. R., Kuhar, M. J., & Pickel, V. M. (1996). The dopamine transporter is localized to dendritic and axonal plasma membranes of nigrostriatal dopaminergic neurons. *The Journal of Neuroscience: The Official Journal of the Society for Neuroscience*, *16*(2), 436–447.

Nishijima, H., & Tomiyama, M. (2016). What Mechanisms Are Responsible for the Reuptake of Levodopa-Derived Dopamine in Parkinsonian Striatum?. *Frontiers in neuroscience*, *10*, 575. doi:10.3389/fnins.2016.00575

Olanow C. W., Obeso J. A. (2000). Preventing levodopa-induced dyskinesias. *Annals of Neurology*. 47(4 Suppl. 1), S167–S176. Discussion: S176–S168.

Ozelius LJ, Senthil G, Saunders-Pullman R, Ohmann E, Deligtisch A, Tagliati M, Hunt AL, Klein C, Henick B, Hailpern SM, Lipton RB, Soto-Valencia J, Risch N, Bressman SB .(2006). LRRK2 G2019S as a cause of Parkinson's disease in Ashkenazi Jews. *The New England Journal of Medicine.* 354:424–425.

Park J, Lee SB, Lee S, Kim Y, Song S, Kim S, Bae E, Kim J, Shong M, Kim JM, Chung J. (2006). Mitochondrial dysfunction in Drosophila PINK1 mutants is complemented by parkin. *Nature*. 441(7097):1157-61.

Polymeropoulos M. H., Lavedan C., Leroy E., Ide S. E., Dehejia A., Dutra A., et al. (1997). Mutation in the alpha-synuclein gene identified in families with Parkinson's disease. *Science*. 276, 2045–2047

Recchia A., Debetto P., Negro A., Guidolin D., Skaper S. D., Giusti P. (2004). Alpha-synuclein and Parkinson's disease. *The FASEB Journal*. 18, 617–626 10.1096/fj.03-0338rev

Ribeiro F. M., Camargos E. R., De Souza L. C., Teixeira A. L. (2013). Animal models of neurodegenerative diseases. *Revista Brasileira de Psiquiatria* . 35(Suppl. 2), S82–S91. 10.1590/1516-4446-2013-1157

Rice, M. E., Patel, J. C., & Cragg, S. J. (2011). Dopamine release in the basal ganglia. *Neuroscience*, *198*, 112–137. doi:10.1016/j.neuroscience.2011.08.066

Saha, S., Guillily, M. D., Ferree, A., Lanceta, J., Chan, D., Ghosh, J., … Wolozin, B. (2009). LRRK2 modulates vulnerability to mitochondrial dysfunction in Caenorhabditis elegans. *The Journal of neuroscience: the official journal of the Society for Neuroscience*, *29*(29), 9210–9218. doi:10.1523/JNEUROSCI.2281-09.2009

Sakaguchi-Nakashima A, Meir JY, Jin Y, Matsumoto K, Hisamoto N. LRK-1, a C. elegans PARK8-related kinase, regulates axonal-dendritic polarity of SV proteins. *Current Biology*. 2007;17:592–598

Sämann, J., Hegermann, J., von Gromoff, E., Eimer, S., Baumeister, R., & Schmidt, E. (2009). Caenorhabditits elegans LRK-1 and PINK-1 act antagonistically in stress response and neurite outgrowth. *The Journal of biological chemistry*, *284*(24), 16482–16491. doi:10.1074/jbc.M808255200

Scatton B., Javoy-Agid F., Rouquier L., Dubois B., Agid Y. Reduction of cortical dopamine, noradrenaline, serotonin and their metabolites in Parkinson's disease.(1983). *Brain Research*. 275(2):321–328. doi: 10.1016/0006-8993(83)90993-9.

Schirinzi T., Madeo G., Martella G., Maltese M., Picconi B., Calabresi P., et al..(2016). Early synaptic dysfunction in Parkinson's disease: insights from animal models. *Movement Disorders.* 31, 802–813. 10.1002/mds.26620

Shimada S, Kitayama S, Lin CL, Pate1 A, Nanthakumar E, Gregor P, Kuhar M, Uhl G (1991) Cloning and expression of a cocaine-sensitive dopamine transporter complementary DNA [published erratum appears in Science (1992) 255:1195]. *Science* 254:576-578.

Shulman JM, De Jager PL, Feany MB. (2011). Parkinson's disease: Genetics and pathogenesis. *Annual Review of Pathology.* 6: 193–222

Simon-Sanchez J, Schulte C, Bras JM, Sharma M, Gibbs JR, Berg D, Paisan-Ruiz C, Lichtner P, Scholz SW, Hernandez DG, et al.(2009).Genome-wide association study reveals genetic risk underlying Parkinson's disease. *Nature Genetics.* 41: 1308–1312

Smith, W. W., Pei, Z., Jiang, H., Moore, D. J., Liang, Y., West, A. B., … Ross, C. A. (2005). Leucine-rich repeat kinase 2 (LRRK2) interacts with parkin, and mutant LRRK2 induces neuronal degeneration. *Proceedings of the National Academy of Sciences of the United States of America*, *102*(51), 18676–18681. doi:10.1073/pnas.0508052102

Spillantini M. G., Schmidt M. L., Lee V. M., Trojanowski J. Q., Jakes R., Goedert M. (1997). Alpha-synuclein in Lewy bodies. *Nature* 388, 839–840. 10.1038/42166

Sulston J., Dew M., Brenner S. (1975). Dopaminergic neurons in the nematode *Caenorhabditis elegans*. *Journal of Comparative Neurology.* 163, 215–226 10.1002/cne.901630207

Taylor T. N., Greene J. G., Miller G. W. (2010). Behavioral phenotyping of mouse models of Parkinson's disease. *Behavioral Brain Research.* 211, 1–10. 10.1016/j.bbr.2010.03.004

Takahashi H, Iwata T, Kitagawa Y, Takahashi RH, Sato Y, Wakabayashi H, Takashima M, Kido H, Nagashima K, Kenney K, Gibbs CJ, Kurata T (1999). Increased levels of epsilon and gamma isoforms of 14-3-3 proteins in cerebrospinal fluid in patients with Creutzfeldt-Jakob disease. *Clinical and Diagnostic Laboratory Immunology*. 6 (6): 983–5.

Thiele S. L., Warre R., Nash J. E. (2012). Development of a unilaterally-lesioned 6-OHDA Mouse Model of Parkinson's disease. *Journal of Visualized Experiments*. Exp. pii:3234 10.3791/3234

Thiruchelvam, M., McCormack, A., Richfield, E.K., Baggs, R.B., Tank, A.W., Di Monte, D.A., and Cory-Slechta, D.A. (2013). Age-related irreversible progressive nigrostriatal dopaminergic neurotoxicity in the paraquat and maneb model of the Parkinson’s disease phenotype. *European Journal of Neruoscience.* 18, 589–600

Tanner C.M. (1992). Occupational and environmental causes of Parkinsonism. *Occupational Medicine.* 7:503–513

Venderova K., Kabbach G., Abdel-Messih E., Zhang Y., Parks R. J., Imai Y., et al. . (2009). Leucine-rich repeat kinase 2 interacts with Parkin, DJ-1 and PINK-1 in a *Drosophila melanogaster* model of Parkinson's disease. *Human Molecular Genetics*. 18, 4390–4404. 10.1093/hmg/ddp394

Vives-Bauza C., Zhou C., Huang Y., Cui M., de Vries R.L., Kim J., May J., Tocilescu M.A., Liu W., Ko H.S., et al. (2010). PINK1-dependent recruitment of parkin to

mitochondria in mitophagy. *Proceedings of the National Academy of Sciences of the United States of America*. 2010;107:378–383.

Warner T. T., Schapira A. H. (2003). Genetic and environmental factors in the cause of Parkinson's disease. *Annals of Neurology.* 53(Suppl. 3), S16–S23; Discussion S23–S15. 10.1002/ana.10487

Winklhofer, K. F., & Haass, C. (2010). Mitochondrial dysfunction in Parkinson's disease. *Biochimica et Biophysica Acta*, *1802*(1), 29–44.

Xiong H., Wang D., Chen L., Choo Y.S., Ma H., Tang C., Xia K., Jiang W., Ronai Z., Zhuang X., et al. Parkin, PINK1, and DJ-1 form a ubiquitin E3 ligase complex promoting unfolded protein degradation. *Journal of Clinical Investigation.* 2009;119:650–660

Yan, M. H., Wang, X., & Zhu, X. (2013). Mitochondrial defects and oxidative stress in Alzheimer disease and Parkinson disease. *Free radical biology & medicine*, *62*, 90–101. doi:10.1016/j.freeradbiomed.2012.11.014

Yao, C., El Khoury, R., Wang, W., Byrd, T. A., Pehek, E. A., Thacker, C., … Chen, S. G. (2010). LRRK2-mediated neurodegeneration and dysfunction of dopaminergic neurons in a Caenorhabditis elegans model of Parkinson's disease. *Neurobiology of disease*, *40*(1), 73–81. doi:10.1016/j.nbd.2010.04.002

Zimprich A, Biskup S, Leitner P, Lichtner P, Farrer M, Lincoln S, Kachergus J, Hulihan M, Uitti RJ, Calne DB, Stoessl AJ, Pfeiffer RF, Patenge N, Carbajal IC, Vieregge P, Asmus F, Muller-Myhsok B, Dickson DW, Meitinger T, Strom TM, Wszolek ZK, Gasser T.(2004). Mutations in LRRK2 Cause Autosomal-Dominant Parkinsonism with Pleomorphic Pathology. *Neuron*. 44:601–607

CURRICULUM VITAE