SURPRISING BEHAVIORAL AND NEUROCHEMICAL ENHANCEMENTS IN MICE WITH COMBINED MUTATIONS LINKED TO PARKINSON'S DISEASE

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DEDICATION

To my family. Forever and Always.

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SURPRISING BEHAVIORAL AND NEUROCHEMICAL ENHANCEMENTS IN MICE WITH COMBINED MUTATIONS LINKED TO PARKINSON'S DISEASE

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SURPRISING BEHAVIORAL AND NEUROCHEMICAL ENHANCEMENTS IN MICE WITH COMBINED MUTATIONS LINKED TO PARKINSON'S DISEASE

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Parkinson's disease (PD) is the second most common neurodegenerative disease, after Alzheimer's disease, afflicting over a million people in the United States alone. PD is an age-dependent disease that causes progressive death of dopamine-producing neurons in the substrantia nigra and depleted dopamine in the striatum. Loss of striatal dopamine results in locomotor symptoms such as bradykinesia, tremor, rigidity and postural instability. Although most forms of the disease are spontaneous, a subset of cases are genetic and humans lacking expression of either *Parkin* or *DJ-1* develop PD. However, one limitation to studying PD is a lack of rodent models that recapitulate both the dopaminergic and motor symptoms as well as the age-dependent development of this disease. In fact, mice deficient for either one or both *Parkin* and *DJ-1* genes have no dopaminergic neuron loss or deficiency in motor abilities. Therefore, I aimed to develop a rodent model of Parkinson's disease that mimics the progressive symptoms observed in humans by crossing mice deficient for two genes causative for PD, *Parkin* and *DJ-1*. I also crossed mice deficient for *Parkin* and *DJ-1* with mice deficient for glutathione peroxidase 1 (*Gpx1*), an antioxidant that is decreased in the brains of PD patients and increased in aged *DJ-1* deficient mouse brains. Instead of the

expected loss of dopamine, *Parkin*$^{-/-}$*DJ-1*$^{-/-}$*Gpx1*$^{-/-}$ mice exhibit increased striatal dopamine while *Parkin*$^{-/-}$*DJ-1*$^{-/-}$ mice have increased serotonin in multiple brain regions. Additionally, motor phenotypes in these mice do not replicate symptoms observed in PD because *Parkin*$^{-/-}$ *DJ-1*$^{-/-}$ mice have an unexpected increase in latency to fall from the rotarod in the absence of other significant behavioral phenotypes. These results led me to examine the levels of proteins related to neurotransmitter synthesis and transport and to test non-motor behaviors in *Parkin*$^{-/-}$*DJ-1*$^{-/-}$ mice. Behavior tests suggest that *Parkin*$^{-/-}$*DJ-1*$^{-/-}$ mice have improved rotarod performance due to cognitive, rather than motor changes.

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PRIOR PUBLICATIONS

Hennis MR, Marvin MA, Taylor CM II, Goldberg MS. Surprising behavioral and neurochemical enhancements in mice with combined mutations linked to Parkinson's disease. Neurobiology of Disease 62C: 113-123, 2013.

Hennis, MR, Seamans KW, Marvin MA, Casey BH, Goldberg MS. Behavioral and Neurotransmitter Abnormalities in Mice Deficient for Parkin, DJ-1 and Superoxide Dismutase. PLOS One. Submitted.

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LIST OF DEFINITIONS

ADD – Attention deficit disorder

AR-JP – Autosomal recessive juvenile parkinsonism

Gpx1 – Glutathione Peroxidase 1

DA – Dopamine

DAB – 3,3'-Diaminobenzidine

DAPI – 4',6-diamidino-2-phenylindole

DBS – Deep brain stimulation

DNP – 2,4-Dinitrophenol

DNPH – 2,4-Dinitrophenylhydrazine

DRN – Dorsal raphe nucleus

DOPAC – 3,4-Dihydroxyphenylacetic acid

FDA – Food and Drug Administration

4-HNE – 4-hydroxynonenal

HPLC – High performance liquid chromatography

HVA – Homovanillic acid

H_2O_2 – Hydrogen peroxide

LC – Locus ceruleus

L-DOPA – Levadopa

LID – L-DOPA induced dyskinesia

MAO – Monoamine oxidase

MAO-B – Monoamine oxidase B

MAOI – Monoamine oxidase inhibitor

MPH – Methylphenidate

MPPP – 1-Methyl-4-phenyl-4-propionoxypiperidine

$MPPP^+$ – 1-Methyl-4-phenylpyridinium

MPTP – 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine

3-MT – 3-methoxytyramine

NSAID – Nonsteroidal anti-inflammatory drug

6-OHDA – 6-hydroxydopamine

8-OHdG – 8-hydroxy-deoxyguanisine

PD – Parkinson's disease

PINK1 – PTEN induced putative kinase 1

PCR – Polymerase chain reaction

SERT – Serotonin transporter

SSRI – Selective serotonin reuptake inhibitor

SOD1 – Superoxide dismutase 1

SOD2 – Superoxide dismutase 2

TH – Tyrosine hydroxylase

TpH – Tryptophan hydroxylase

WT – Wild type

MAOI – Monoamine oxidase inhibitor

MPH – Methylphenidate

MPPP – 1-Methyl-4-phenyl-4-propionoxypiperidine

CHAPTER ONE
Introduction

BACKGROUND ON PARKINSON'S DISEASE

Parkinson's Disease

Parkinson's disease (PD) is the second most common neurodegenerative disorder currently afflicting 1% of the population between ages 65 and 69, with prevalence increasing to 4% after 70 years of age (de Lau and Breteler, 2006). Descriptions of disease symptoms appear in ancient texts as early as 1000 BCE, although the disease was not described in medical journals until 1817 when James Parkinson published "An Essay on the Shaking Palsy." The four cardinal symptoms of the disease, as described by Parkinson, are bradykinesia, resting tremor, postural imbalance and muscle rigidity. Motor symptoms are caused by the loss of dopamine (DA) in the striatum due to the death of neurons in the substantia nigra (SN), but the cause of this neuronal death is still unknown. Other neurotransmitters, such as serotonin, may also play a role in the development of disease symptoms. Currently, treatments for symptoms include levadopa (L-DOPA), monoamine oxidase inhibitors (MAOIs) and deep brain stimulation, but no cure exists.

History

Some of the earliest written descriptions of disorders resembling PD can be found in ancient Chinese and Indian texts dating back to 1000 BCE (Goetz, 2011). Parkinson's disease was first described in a medical journal in 1817 by James Parkinson, the man whose name was eventually given to the disease, in "An Essay on the Shaking Palsy" (Parkinson, 2002,

Kempster et al., 2007). Parkinson's initial description of the disease was later clarified by Jean-Marie Charcot who made the distinction between PD and other disorders with similar symptoms, defining PD as a distinctive disorder (Louis, 1997). In the early 1900s, Konstantin Tretiakoff and Rolf Hassler found nigral cell loss to be causative for this disease (Lees et al., 2008, Parent and Parent, 2010) and in 1988, pigmented dopaminergic neurons were determined to be selectively susceptible to death in PD (Hirsch et al., 1988). In the mid-1900s Arvid Carlsson discovered that dopamine was a neurotransmitter in its own right which was substantiated by Herbert Ehringer and Oleh Hornykiewicz who determined that dopamine levels were lower in the brains of PD patients than controls (Ehringer and Hornykiewicz, 1960, Yeragani et al., 2010). As a result of these findings, administration of L-DOPA, the molecular precursor to dopamine, was found to be therapeutically beneficial in PD patients and has since been considered the "gold-standard" in treating PD.

In 1973, Hans Bernheimer hypothesized that compensatory changes may occur in early disease stages, resulting in a brief period of increased dopamine and/or serotonin prior to the loss of neurotransmitters later in the course of the disease. Furthermore, it has been suggested that neurological changes may occur outside of the basal ganglia (Jellinger, 1991, Blandini, 2013). However, this is not yet a widely accepted theory, and lacks specific evidence.

Symptoms

The four cardinal symptoms of PD are resting tremor, bradykinesia (slowness of movement), muscle rigidity and postural instability. Resting tremor was famously first identified by

Parkinson (Kempster et al., 2007), although his writing alludes to other motor symptoms as well. Charcot later defined bradykinesia as a motor symptom in addition to the classical PD tremor (Goetz, 1986). In addition to these main symptoms, non-motor symptoms have been identified as well, including anosmia, which is not correlated to disease severity yet is reported to occur in a high number of PD patients (Doty et al., 1988, Doty et al., 1992), and reduced cognitive abilities (Levin et al., 1989, Mahieux et al., 1998, Levy et al., 2002a, Levy et al., 2002b). Cognitive symptoms are present in approximately 40% of all PD cases (Emre, 2004) and are typically found in the later stages of the disease, although there are reports of decreased cognition at earlier stages (Lees and Smith, 1983, Levin et al., 1989). Most non-motor symptoms are neuropsychiatric, such as depression and anxiety disorders, or autonomic, such as sleep disorders (Lindgren and Dunnett, 2012, Bonnet and Czernecki, 2013). These mental deficits include difficulty with set-shifting and greater perseverative errors in cognitive tasks, which have been hypothesized to indicate increased mental rigidity in PD patients (Lees and Smith, 1983). Furthermore, cognitive deficits in the early stages of the disease may be independent of dopamine deficiency (Cooper et al., 1991, Bonnet and Czernecki, 2013), and exacerbation of these symptoms has been shown to level-off, even as motor abilities continue to deteriorate (Pavao et al., 2012). As a result, some have suggested that the pathways linking the cortex to the basal ganglia are instrumental in disease development, in contrast to the theory that the entirety of the disease occurs in the nigrostriatal pathway (Lindgren and Dunnett, 2012).

Differences in the onset and development of cognitive symptoms suggest the involvement of other brain regions and neurotransmitter systems. Cognitive deficiencies can

appear at any stage of the disease, although they are most common at older ages (Lees and Smith, 1983, Levin et al., 1989). Zurkovsky et al. (2013) found that a cholinergic deficit in addition to the dopaminergic deficit is responsible for the formation of cognitive symptoms. The cognitive functions that are most often affected in PD include executive functions and memory although it should be noted that memory problems in PD differ from the amnesia of Alzheimer's disease in that memories can be formed, but are not easily retrieved (Emre, 2004). Furthermore, dementia in PD has been linked to age of onset, with older onset patients exhibiting dementia faster than early-onset patients (Halliday and McCann, 2010). Additionally, underlining the difference between PD and diseases such as Alzheimer's and other forms of dementia, the cognitive dysfunctions found in PD can be present in the absence of dementia. Finally, there are conflicting reports on some cognitive deficiencies in PD. For example attention has been reported as both affected and not affected in PD (Levin et al., 1989, Emre, 2004).

Pathology

Parkinson's disease is defined neuropathologically by a loss of dopamine-producing neurons in the SN accompanied by intracellular neuronal inclusions known as Lewy bodies (Braak et al., 2003). Lewy bodies are abnormal protein aggregates composed of alpha-synuclein and other proteins, such as ubiquitin. Often, Lewy bodies are present in patients with dementia and it has been hypothesized that a greater number of alpha synuclein inclusions is related to the severity of disease symptoms (Wakabayashi, 2013) although contradictory evidence makes these findings difficult to interpret (Halliday and McCann, 2010).

Typically, it is thought that loss of these neurons leads to a denervation of dopaminergic terminals in the striatum and a decrease in the striatal dopamine levels. An alternative hypothesis claims that denervation of dopaminergic terminals occurs prior to the death of nigral neurons, leading to a retrograde neurodegeneration of dopaminergic axons, eventually leading to nigral neuron death and PD (Bernheimer et al., 1973, Pham et al., 2012). Overall, there is a correlation between the severity of dopaminergic neuron loss and length of disease although additional correlations, such as presence of Lewy bodies, are less definitive due to the pathological variation present in PD (Halliday and McCann, 2010).

In addition to neuronal loss in the SN, there are multiple reports of neuron loss in other areas of the brain, particularly in the locus ceruleus (LC) and dorsal raphe nucleus (DRN), which have higher production of noradrenaline and serotonin, respectively (Jellinger, 1991). In fact, it has been reported that loss of neurons occurs in the LC early in the disease process (Szot et al., 2012).

Due to the wide range of pathological symptoms that can occur in PD, Braak et al (2003) have published definitions of stages of idiopathic PD. Although these stages were described for sporadic PD, the disease mechanisms are thought to be involved in familial PD as well (Fitzmaurice et al., 2003). These stages are defined as starting with alpha-synuclein immunopositive Lewy bodies and lesions in the dorsal motor nucleus followed by deterioration in the cortical areas, sensory and motor areas (Braak et al., 2003). However, concerns have been raised in response to this report over whether these stages accurately define sporadic PD, specifically because the stages do not necessarily correlate with the expected presence of core motor symptoms, later stages of the disease do not correspond to

older patients, and the presence of Lewy bodies does not always indicate the presence of PD (Halliday and McCann, 2010). Development of PD is highly variable, complicating interpretations of results relating to disease progression.

Molecular Causes

The underlying causes of PD remain unknown, but the main hypotheses in the field involve oxidative stress, mitochondrial dysfunction, or both. Mitochondrial damage was first identified as a possible cause of PD in the 1980s following development of PD-like symptoms resulting from 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) poisoning in drug users (Langston et al., 1983, Blandini, 2013). MPTP is a contaminant of 1-Methyl-4-phenyl-4-propionoxypiperidine (MPPP), an opioid that is often used as a recreational drug. When MPTP crosses the blood-brain barrier, it is converted to MPP^+ by monoamine oxidase B (MAO-B), which is then transported into dopamine neurons of the SN by the dopamine transporter (DAT) and inhibits mitochondrial complex I activity, ultimately resulting in neuronal death. Damage to mitochondria and to mitochondrial dynamics causes increased production of reactive oxygen species (ROS), which then causes oxidative damage within the cell.

Evidence of oxidative damage in PD and animal models of PD has been reported in the form of increased lipid peroxidation, protein carbonylation, increased 4-hydroxynonenal (4-HNE), increased markers of DNA damage such as 8-hydroxy-deoxyguanisine (8-OHdgG), and increased iron in the SN (Dexter et al., 1989a, Dexter et al., 1989b, Fahn and Cohen, 1992, Spencer et al., 1994, Yoritaka et al., 1996, Seet et al., 2010). Mice deficient for

DJ-1, a gene of uncertain function, loss of which causes PD in humans, have decreased protection from mitochondrial uncoupling and increased mitochondrial reactive oxygen species production, which typically leads to increased oxidative damage (Guzman et al., 2010). Additionally, the dopaminergic cells of the SN are exposed to exceptionally high levels of ROS due to high levels of cytoplasmic dopamine, which is known to autoxidize and can be toxic (Fahn and Cohen, 1992, Hattoria et al., 2009, Blandini, 2013).

Other candidates for causative mechanisms in PD include protein aggregation and neuroinflammation. Support for protein aggregation as a cause of PD comes from the presence of Lewy bodies formed from aggregated alpha-synuclein in the brains of PD patients, as well as mutations in alpha-synuclein that have been causally linked to dominantly inherited familial PD (Polymeropoulos et al., 1997). Neuroinflammation has been suggested to play a role in PD because administration of either MPTP or 6-hydroxydopamine (6-OHDA) results in various inflammatory responses such as vascular permeability or microglial activation (Blandini, 2013). Furthermore, mice deficient for *Parkin*, an E3 ubiquitin ligase believed to be involved in mitochondrial autophagy (Shimura et al., 2000), have been found to have increase susceptibility to inflammatory insults (Frank-Cannon et al., 2008), and microglial inhibition is protective against MPTP neurotoxicity (Blandini, 2013).

Finally, it is possible that other system disruptions, such as iron homeostasis, can play a role in the development of PD. Increased iron has been observed in the brains of PD patients, although whether this is a cause or effect of PD is unclear. Iron is required for many normal cellular processes but excessive iron can be toxic due to its reactive nature, which can lead to the generation of ROS and increased oxidative stress (Kaur and Andersen, 2004).

This theory, although somewhat controversial, is supported by work demonstrating the effects of iron on mitochondrial homeostasis (Xu et al., 2013), as well as the involvement of iron in the metabolism of dopamine and its reactivity with hydrogen peroxide (H_2O_2) (Kaur and Andersen, 2004).

Realistically, all of these potential causes may be involved in a common pathway in PD (Figure 1-1), such that mitochondrial dysfunction creates reactive oxygen species that lead to oxidative stress, which can contribute to alpha-synuclein aggregation and microglial activation. In turn, alpha-synuclein and reactive oxygen species are also thought to contribute to the development of mitochondrial dysfunction (Khan et al., 2012), creating a feedback loop in which mechanisms leading to the development of PD spiral out of control.

Treatments

L-DOPA

There is no cure for PD. Current treatment cannot slow or stop the progression of the disease, nor can they reverse neuronal loss in PD. Instead, the goal of all current treatments is to improve disease symptoms. Some of the earliest treatments for PD were anticholinergic drugs developed by Charcot (Goetz, 2011), although use of these has largely declined following the discovery of L-DOPA, which is now considered to be the "gold standard" of PD treatment. Initially, L-DOPA was thought to be biologically inactive, following its discovery in legumes in 1913 (Hornykiewicz, 2002b). However, it was later found to act as a vasopressor, prior to being discovered in striatal samples. In the 1960s, dopamine was found

to be depleted in the brains of PD patients, implicating, for the first time, that it may be involved in the disease process. Ultimately, because L-DOPA is a precursor to dopamine production and is able to pass through the blood-brain barrier, a 1969 double-blind study determined that L-DOPA was an effective treatment for PD (Hornykiewicz and Kish, 1987, Hornykiewicz, 2002a).

Monoamine Oxidase Inhibitors

Other treatments for PD have been developed, including the use of MAO-B inhibitors and deep brain stimulation (DBS). MAO-B inhibitors, such as selegeline and rasagiline, prevent the metabolism of amines including noradrenaline, adrenaline, and most importantly dopamine (Riederer and Laux, 2011), thus increasing the levels of dopamine within the brain and relieving many of the disease motor symptoms, both alone and when used in conjunction with administration of L-DOPA (Riederer and Laux, 2011). Older, irreversible MAOIs were known

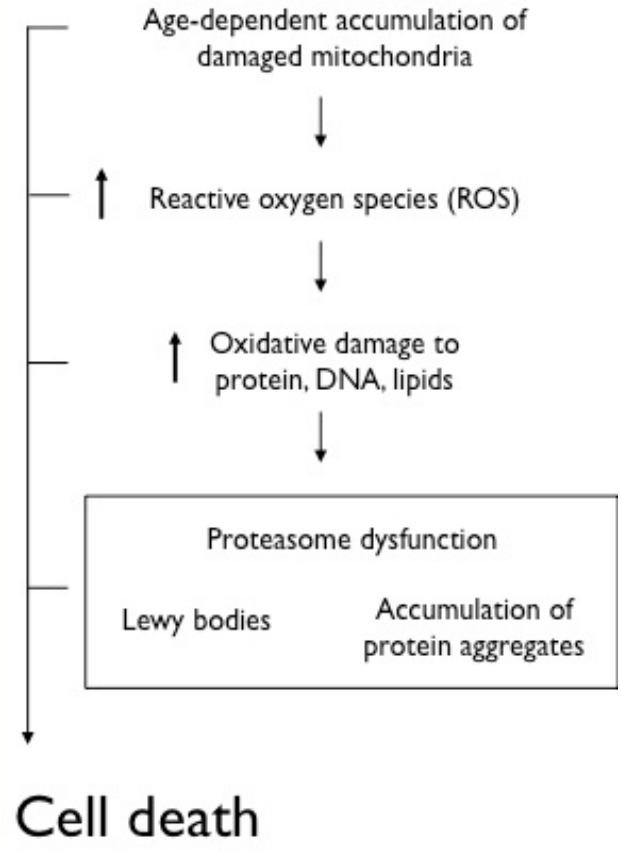

Figure 1-1. Depiction of the mechanisms causative for oxidative stress in dopaminergic nigral neurons. Accumulated mitochondrial damage leads to increased reactive oxygen species, oxidative damage and protein accumulation. Alternatively, each downstream even may occur independently and lead to PD.

to cause undesirable side effect such as the "cheese effect" (an increase in blood pressure due to the MAOI inhibiting the breakdown of diet-derived biogenic amines) and for restoring serotonin and noradrenaline levels in the absence of an effect on dopamine (Riederer and Laux, 2011, Park et al., 2013). In more recent years, however, selective, reversible MAOIs have been developed, with fewer side effects (Riederer and Laux, 2011).

Deep Brain Stimulation

Deep brain stimulation is the targeted electrical stimulation of specific nodes in the basal ganglia circuitry used as treatment in patients with motor symptoms that are difficult to manage and do not respond well to L-DOPA and other non-invasive therapies (Duker and Espay, 2013). DBS involves targeting basal ganglia structures such as the subthalamic nucleus or globus pallidus, a rationale that is supported by experimental evidence pointing to lesions in the basal ganglia as causative for parkinsonian symptoms (Breit et al., 2004). The DBS procedure was initially pioneered in the 1990s by Alim-Louis Benabid, with unilateral DBS approved by the Food and Drug Administration (FDA) in 1997 and bilateral DBS approved in 2002 (Benabid et al., 1998). In fact, bilateral DBS has been found to be a more effective treatment than the "best medical treatment," a combination of managed pharmacological treatment and nonpharmacological therapy, offered at Veterans Affairs and university hospitals (Weaver et al., 2009). Although DBS has been shown to be an effective treatment, especially for advanced PD, this treatment does have several adverse effects that are primarily neurocognitive (Benabid et al., 2009).

The disadvantage of these treatments is that they work by amplifying the signal produced by the nigral dopaminergic neurons and therefore require a sufficient number of surviving nigral neurons in order to be effective. Over time, these treatments lose their effectiveness due to the progressive loss of nigral neurons. Therefore, there remains a need to better understand the disease process of PD, particularly the underlying causes of dopamine neuron loss.

Neurotransmitters in Parkinson's Disease

Dopamine

Loss of striatal dopamine is the accepted explanation for motor dysfunction in PD (Scatton et al., 1983). This is supported by the effectiveness of dopamine replacement therapies as treatment for motor symptoms, including administration of L-DOPA, inhibition of monoamine oxidase (MAO), and deep brain stimulation, which activates the striatal circuits that would normally be activated by dopamine neurotransmission (Hornykiewicz and Kish, 1987, Hornykiewicz, 2002b, a, Hoglinger et al., 2004). In fact, mutations in the tyrosine hydroxylase (TH) gene, the rate limiting enzyme in dopamine production, have been reported to result in parkinsonian symptoms (Bademci et al., 2012), and it has been reported that the pigmented dopaminergic neurons are particularly susceptible to neurodegeneration, which may be one explanation for why these neurons specifically die in PD (Hirsch et al., 1988). Some studies have linked the Parkinson's related gene *Parkin* to dopaminergic effects, such as enhancing surface expression of dopamine transporter (DAT) (Jiang et al., 2004, Jiang et al., 2012). However, more recent advances in the study of PD have indicated that dopamine

loss is unlikely to be the whole story behind PD pathology (Curzon, 1977, Scatton et al., 1983, Jellinger, 1991, Devos et al., 2010).

Serotonin

Ergot and ergot-derived drugs, which act on the serotonergic system, have also been used to treat PD. Ergot is a type of fungus that can cause symptoms such as extreme vasoconstriction, hallucinations, nausea, seizures, and unconsciousness. However, in smaller doses, derivatives of ergot can be medicinally beneficial (Eadie, 2003). The serotonergic system is known to be affected in PD (Bedard et al., 2011), especially in the early stages, and is affected in animal models of PD as well (Rampersaud et al., 2012). In particular, compensation, such as serotoninergic sprouting, has been observed in rat brains with dopaminergic lesions (Zhou et al., 1991, Maeda et al., 2003, Maeda et al., 2005), dopamine-depleted mouse brains (Li et al., 2013) and MPTP-treated monkeys that have increased serotonin in symptomatic striatum compared to unaffected striatum (Gaspar et al., 1993, Boulet et al., 2008). PTEN induced putative kinase 1 (*PINK1*) deficiency in mice results in decreased serotonin innervation of the olfactory bulb as well as impaired gait and olfaction (Glasl et al., 2012). Furthermore, serotonin transporter (SERT) expression is reduced in PD patients (Roselli et al., 2010), SERT knockout mice have decreased rotarod performance and general motor deficits (Morelli et al., 2011), and these effects are replicated in mice treated with chronic fluoxetine, a selective serotonin reuptake inhibitor (SSRI) (Lee, 2012). Additionally, SERT knockout mice have been shown to exhibit antidepressant-like behavioral phenotypes and reduced aggression (Holmes et al., 2002a, Holmes et al., 2002b),

which is to be expected in mice that would presumably have higher levels of synaptic serotonin due to a loss of the protein responsible for reuptake. Additionally, the mitochondrial complex I inhibitor rotenone has been shown to kill serotonergic neurons (Ren and Feng, 2007), although MPTP has incongruently been shown to increase the numbers of serotonergic neurons (Lesemann et al., 2012). In fact, extensive evidence suggests that, following induction of dopaminergic lesions by MPTP or 6-OHDA in both rats and mice, serotonin can compensate for dopamine loss and dopaminergic lesions can be hyperinnervated by serotonergic projections (Zhou et al., 1991, Kostrzewa et al., 1998, Rozas et al., 1998, Maeda et al., 2003, Brown and Gerfen, 2006, Zeng et al., 2010, Li et al., 2013). Some studies suggest that serotonergic fibers contribute to dopamine production following L-DOPA administration in denervated striatum (Maeda et al., 2005). Furthermore, postmortem studies indicate that in late-stage PD brains, there is a decrease in striatal serotonergic innervation (Raisman et al., 1986, Bedard et al., 2011), indicative of potential serotonergic involvement in PD, possibly depriving the brain of a compensatory response to loss of dopamine (Kish et al., 2008, Li et al., 2013). The loss of serotonin at later stages of the disease does not fit with the increased serotonergic production and innervation, as observed in the studies described above. However, this may be due to the timing of the disease onset and progression.

The dopaminergic and serotonergic systems interact within the striatum (Devos et al., 2010) where the serotonin-releasing dorsal raphe nucleus (DRN) innervates the striatum along with the SN. In fact, evidence suggests that postsynaptic 5-HT2c receptors in the striatum are responsible for increased firing rates and elevated striatal dopamine baseline

levels (Mathur and Lovinger, 2012). However, this is often overlooked in studies of PD because the extent of damage to the serotonergic system in PD small compared to the dopaminergic system, and serotonin has remained of interest in the Parkinson's field primarily due to the link between serotonin and L-DOPA induced dyskinesias (LID) (Mathur and Lovinger, 2012).

The serotonin system, however, is not the only non-dopaminergic neurotransmitter system to be affected in PD. Some of the earliest degeneration in PD occurs in noradrenergic neurons, particularly in the locus coeruleus (Devos et al., 2010). Similarly to serotonin, norepinephrine also innervates the striatum but exhibits a lower proportion of neuron loss in PD compared to the dopaminergic system. Involvement of both the serotonin and noradrenergic systems in PD is indicative of wider involvement by monoamine neurotransmitters beyond dopamine and suggests that by focusing solely on dopamine, the field of PD research may be missing clues to the origins of the disease or to potential treatments.

CHAPTER TWO
Review of the Literature

CAUSES OF PARKINSON'S DISEASE

Idiopathic vs Familial Parkinson's Disease

The majority of PD cases are sporadic, however 5-10% of cases can be traced to genetic causes. Five genes have been identified as causative for PD. Mutations in two of these genes, *alpha-synuclein* and *leucine-rich repeat kinase 2* (*LRRK2*), are dominantly inherited, while the remaining three, *Parkin*, *DJ-1*, and *PINK1*, are recessively inherited, representing loss-of-function mutations. Sporadic and familial PD may share common disease pathways (Figure 1-1), suggesting that an understanding of the mechanisms involved in familial PD development may lead to a better understanding of the sporadic form.

Environmental Causes of Parkinson's Disease

Although no causes of sporadic PD have been definitively identified, some epidemiological studies have indicated that environmental toxins such as pesticides, solvents and metals may be involved in disease development (Goldman, 2013, Kieburtz and Wunderle, 2013). In contrast, there is also data suggesting that solvent and metal exposure does not affect PD risk (Tanner et al., 2009). Many other factors, including gender, brain injury, exercise and use of tobacco, nonsteroidal anti-inflammatory drugs (NSAIDs) and caffeine may also affect the incidence of PD (Kieburtz and Wunderle, 2013).

Pesticides

Pesticides can act by causing oxidative or mitochondrial stress, and thus may influence the development of PD in humans with regular exposure to these toxins. Living in rural areas, well-water use, and farming have all been associated with PD risk (Goldman, 2013). As a result, certain pesticides such as paraquat and rotenone have been used to develop PD models in mice and rats. Rotenone, an insecticide, has a mechanism of action similar to MPTP because it is known to inhibit Complex I of the mitochondrial electron transport chain. J. Timothy Greenamyre was the first to demonstrate that administration of rotenone to rodents recapitulated symptoms of PD, including nigral neuron loss and rigidity (Betarbet et al., 2000). Rotenone has since been shown to cause reproducible selective nigral neuron loss and dopamine depletion as well as loss of dopaminergic terminals and alpha-synuclein inclusions (Cannon et al., 2009).

Paraquat, a widely used herbicide, has been commercially available for over 50 years (Goldman, 2013). Although some case-control studies indicate the involvement of paraquat in the development of PD, other studies refute this claim (Tanner et al., 2009, Firestone et al., 2010, Tanner et al., 2011). Similar to MPTP, paraquat can be taken up into dopaminergic terminals following its conversion to paraquat^{+}, where it produces superoxide and causes features of PD in animal models including lipid peroxidation, decreased antioxidant levels, alpha-synuclein aggregation, impaired mitochondrial function, and the selective death of nigral neurons (Brooks et al., 1999, McCormack et al., 2005, Goldman, 2013).

The role of additional factors affecting the development of PD, including solvent and metal exposure, are less clear. Solvents appear to be associated with parkinsonian symptoms according to epidemiological studies (Uitti et al., 1994, Goldman et al., 2012) while exposure to metals, such as iron and lead, has been found to cause related symptoms in experimental animal models (Kala and Jadhav, 1995, Weisskopf et al., 2010, Goldman, 2013). However, solvent exposure causes acute parkinsonism, rather than causing symptoms following a prolonged period of exposure (Uitti et al., 1994). Furthermore, the epidemiological evidence for each remains contested and unclear (Segal, 2012, Goldman, 2013).

Protective Factors

Several lifestyle factors seem to have a protective effect against the development of PD. Interestingly, these include smoking and caffeine (Tanner et al., 2009) as well as exercise and the use of nonsteroidal anti-inflammatory drugs (Goldman, 2013). In fact, administration of purines such as nicotine and caffeine has been found to be neuroprotective in animal models of the disease (Kieburtz and Wunderle, 2013). Potential mechanisms behind these neuroprotective effects are thought to come from alterations of the dopamine receptors, as in the case of nicotine or from the antagonizing effects of caffeine on adenosine A2 receptors (Kachroo et al., 2010, Garcia-Montes et al., 2012). Recent research into the effects of purines on PD has included urate in addition to caffeine and adenosine, and has suggested therapeutic uses for these molecules (Chen et al., 2012).

Genetic Causes of Parkinson's Disease

Most cases of PD are apparently sporadic, and the underlying cause of the nigral neuron loss is unknown. However, up to 10% of all cases are caused by genetic mutations, although many other cases may have underlying genetic factors despite occurring sporadically (Warner and Schapira, 2003). Five genes have previously been identified as causative for familial PD (Dawson et al., 2010, Horowitz and Greenamyre, 2010, Lopez and Sidransky, 2010, Corti et al., 2011, Hattori, 2012, Varcin et al., 2012). Mutations in *alpha-synuclein* and *LRRK2* are considered to be dominantly inherited (Puschmann, 2013). A point mutation in *alpha-synuclein* was initially identified as a dominantly inherited cause of PD when individuals in four separate Italian and Greek families were found to have an A53T mutation in conjunction with a PD diagnosis (Nussbaum and Polymeropoulos, 1997, Polymeropoulos et al., 1997). Individuals with the G2019S LRRK2 mutation in families from two separate studies were also found to have PD (Paisan-Ruiz et al., 2004, Zimprich et al., 2004, Di Fonzo et al., 2005, Nichols et al., 2005), although other, rarer PD-causing mutations also exist in this gene (Puschmann, 2013). The normal function of alpha-synuclein remains to be defined, although it may play a role in membrane remodeling and exocytosis (Bendor et al., 2013). LRRK2 is known to be a cytoplasmic kinase that may interact with alpha-synuclein and affect neuronal morphology (Paisan-Ruiz et al., 2004, Zimprich et al., 2004, Guerreiro et al., 2013, Paus et al., 2013).

Mutations in *Parkin*, *DJ-1* and *PINK1* are causally linked to autosomal recessive forms of parkinsonism (Puschmann, 2013). Homozygous mutations in the *Parkin* gene, which are causative for autosomal recessive juvenile parkinsonism (AR-JP) were first discovered in a Japanese family (Hattori et al., 1998, Kitada et al., 1998). Additional

mutations in the *Parkin* gene causative for autosomal recessive parkinsonism have since been described as well (Abbas et al., 1999) and have shown that brains of AR-JP patients do not express Parkin even though this protein is not decreased in the brains of sporadic PD patients (Shimura et al., 1999). Parkin is an E3 ubiquitin ligase found throughout the brain and body (Stichel et al., 2000, Kuhn et al., 2004) that may modulate proteasome activity (Shimura et al., 2000, Um et al., 2010). Therefore, Parkin may be involved in clearing damage due to reactive oxygen species. Given protein aggregation is a pathological feature of PD (Dodson and Guo, 2007), a major hypothesis of PD pathology is that loss of Parkin may contribute to aberrant protein accumulation. Many potential substrates of Parkin have been reported, including the Pael receptor (Omura et al., 2006), mitochondrial hexokinase HKI (Okatsu et al., 2012), miro (Wang et al., 2011a) and Septin 4 (Munoz-Soriano et al., 2012), and some of these putative *Parkin* substrates have been shown to accumulate or cause dysfunction in the absence of *Parkin* (Mandillo et al., 2013). However, in spite of studies indicating that alterations in the expression of these substrates can result in neuronal loss and other problems (Wang et al., 2008), none of these proteins has been identified as a key component of the mechanism of PD development. In addition to ubiquitination of the substrates listed here, *Parkin* may also function in mitophagy, the removal of damaged mitochondria by autophagy (Ding et al., 2012, Vincow et al., 2013). Because mitochondrial deficits can cause an increase in the release of reactive oxygen species, expression of Parkin would be protective against damage due to these deficits.

The absence of another protein, DJ-1, is another protein also results in parkinsonism. *DJ-1* deficiency was identified as a cause of PD through genetic mapping in two families

with autosomal recessive early onset parkinsonism (Bonifati et al., 2003). DJ-1 exists as a dimer, although the L166P mutation found in PD (Abou-Sleiman et al., 2004) prevents this dimerization (Olzmann et al., 2004). Many different functions have been attributed to DJ-1, including roles in transcriptional regulation resulting in regulation of tyrosine hydroxylase (Ishikawa et al., 2010), synaptic membrane function (Usami et al., 2011), protease activity and mitochondrial regulation (Ariga et al., 2013). Most importantly, however, multiple studies have determined *DJ-1* to be neuroprotective (Canet-Aviles et al., 2004), possibly due to antioxidant activity as evidenced by a study that identified DJ-1 as an atypical peroxidase (Andres-Mateos et al., 2007). Although little evidence suggests that DJ-1 plays a direct role in mitochondrial maintenance, it has been implicated in preventing oxidative damage to cells (Dodson and Guo, 2007), and may function as a sensor for oxidative stress (Trempe and Fon, 2013). Regardless of the specifics of DJ-1 function, the activity of this protein is thought to be regulated by the oxidative status of cysteine 106, a residue found to be oxidized in patients with sporadic PD; still it remains unclear whether this is a cause or an effect of the disease (Ariga et al., 2013).

In addition to *Parkin* and *DJ-1*, loss-of-function mutations in *PINK1* have been causally linked to familial PD. PINK1 is a mitochondrial protein kinase whose activity is required for *Parkin*-dependent mitophagy (Valente et al., 2001, Valente et al., 2002, Trempe and Fon, 2013). *PINK1*-deficient mice are more susceptible to MPTP (Haque et al., 2012) and have been reported to have gait and olfactory dysfunction as well as impaired olfactory bulb serotonergic innervation (Glasl et al., 2012) in contrast to earlier studies indicating that

triple mutant mice deficient for *Parkin*, *DJ-1* and *PINK1* do not have classical parkinsonian phenotypes (Kitada et al., 2009)

Induced Animal Models of Parkinson's Disease

Most animal models of PD involve the acute administration of toxins that selectively affect dopaminergic nigral neurons. These toxins include MPTP, rotenone, 6-OHDA, paraquat and other pesticides. Historically, these models have been used to study the effects of nigral cell loss and striatal dopamine depletion, as well as for testing the effectiveness of various potential therapeutics. Upon systemic administration, MPTP is converted to the toxic metabolite MPP+ by astrocytes and then taken up by DAT, allowing it to enter dopaminergic neurons where it then inhibits Complex I function in the mitochondria (Schober, 2004). Intracranial injection of 6-OHDA (a hydroxylated analog of the dopamine molecule) is taken up by dopaminergic neurons and causes oxidative damage, leading to dopaminergic lesions. Both of these neurotoxin models result in loss of nigral neurons and striatal dopamine and some of the motor symptoms (akinesia and tremor in the MPTP model, rotations in the unilateral 6-OHDA model) (Schober, 2004). Each of these has been used successfully in rodents (Lesemann et al., 2012, Zurkovsky et al., 2013) while MPTP is also used in primate models of the disease (Boulet et al., 2008). More recently, additional models using complex I inhibitors have been developed. Paraquat, an herbicide that is structurally similar to MPTP (Brooks et al., 1999), and rotenone, a common pesticide that produces some parkinsonian symptoms in rats (Cannon et al., 2009). These two pesticides have been implicated in PD by epidemiological studies on environmental factors (Tanner et al., 2011), but do not necessarily

recapitulate PD symptoms in animals (Hoglinger et al., 2006). In each of these induced PD models, the problem remains that toxin-induced parkinsonism occurs acutely after toxin exposure whereas PD develops progressively over the course of many decades in humans (Schober, 2004). Therefore, in contrast to neurotoxin models, genetic animal models based on mutations causative for PD have been developed in the hope of creating models that more faithfully reproduce the both the spontaneous age-dependent neurodegeneration and the underlying neuropathological mechanisms of PD.

Genetic Animal Models of Parkinson's Disease

Parkin

Parkin-deficient *Drosophila melanogaster* have dopaminergic neuron loss (Whitworth et al., 2005). In contrast, despite a significant amount of effort and a long list of mild phenotypes, *Parkin*$^{-/-}$ mice are not a good model of PD (Perez and Palmiter, 2005) because they do not exhibit either neuron loss or decreases in dopamine and the reported behavioral phenotypes have been minor. However, multiple studies have found that *Parkin*$^{-/-}$ mice have behavioral deficits associated with PD, including an increased number of slips on a beam walking test in aged mice, reduced exploratory behavior, cognitive deficits and alterations in posture (Zhu et al., 2007). In addition, *Parkin*$^{-/-}$ mice display non-behavioral PD phenotypes, including increased extracellular dopamine, although total striatal dopamine content is unchanged and there is no apparent change in the expression levels of *Parkin* substrates (Goldberg et al., 2003, Itier et al., 2003, Rodriguez-Navarro et al., 2007). Interestingly, *Parkin* overexpression has been found to increase the surface expression of the DAT and thus increase dopamine

uptake (Jiang et al., 2004), which may account for the increased extracellular dopamine in *Parkin*$^{-/-}$ mice. Additionally, *Parkin* has been found to be involved mitochondrial biogenesis (Kuroda et al., 2006) and *Parkin*$^{-/-}$ mice have decreased mitochondrial respiration in the absence of changes in mitochondrial morphology (Palacino et al., 2004). A separate study reports altered mitochondrial function and morphology in *Parkin*-mutant fibroblasts (Mortiboys et al., 2008), although the different conclusions of these studies may be due to differences between human cells and whole organisms. Furthermore, *Parkin* is selectively recruited to impaired mitochondria (Narendra et al., 2008). *Parkin*$^{-/-}$ mice are more susceptible to inflammatory insults (Frank-Cannon et al., 2008) and some toxins such as rotenone (Casarejos et al., 2006), but not 6-OHDA or MPTP (Perez et al., 2005). These mice also have significantly increased glutathione peroxidase 1 (Gpx1) activity, suggesting that loss of *Parkin* may lead to compensatory upregulation of antioxidant activity (Rodriguez-Navarro et al., 2007). Overexpression of *Parkin* has consistently been found to be neuroprotective against various toxins, including MPTP and 6-OHDA, and alpha synuclein expression (Lo Bianco et al., 2004, Vercammen et al., 2006, Ulusoy and Kirik, 2008, Bian et al., 2012).

DJ-1

Expression of DJ-1 is known to increase in cells exposed to oxidative damage (Lev et al., 2008). *DJ-1*$^{-/-}$ cell lines, mice, *Drosophila* and rats have all been shown to be more susceptible to mitochondrial toxins such as rotenone, 6-OHDA and MPTP, although some data indicate that this protective effect may be due to astrocyte interactions with neurons

rather than autonomous effects in neurons (Meulener et al., 2005, Pisani et al., 2006, Mullett and Hinkle, 2009, Aleyasin et al., 2010, Mullett and Hinkle, 2011, Lev et al., 2013). Overexpression of *DJ-1* in human cell lines, *Drosophila*, and mice is neuroprotective (Zhou and Freed, 2005, Hayashi et al., 2009, Junn et al., 2009) against toxins (such as MPTP and 6-OHDA), oxidative stressors (such as H_2O_2), endoplasmic reticulum stressors (such as tunicamycin) and proteasome inhibition, but not pro-apoptotic stressors (such as staurosporin) (Yokota et al., 2003, Taira et al., 2004, Kim et al., 2005, Menzies et al., 2005, Meulener et al., 2005, Yang et al., 2005, Zhang et al., 2005, Andres-Mateos et al., 2007, Gao et al., 2012, Haque et al., 2012). Similar to *Parkin*$^{-/-}$ mice, *DJ-1*$^{-/-}$ mice exhibit no changes in total dopamine levels. However, *DJ-1* deficient mice have increased H_2O_2 production, increased glutathione peroxidase activity and an increase in levels of both Gpx1 and glutathione (Zhou and Freed, 2005, Andres-Mateos et al., 2007). Oxidation of *DJ-1*$^{-/-}$ occurs following a decrease in glutathione (GSH) levels (Miyama et al., 2011). Furthermore, overexpression of *DJ-1* decreases Bax expression, thus inhibiting caspase activation, while the loss of *D-1* increases Bax levels and caspase activity (Fan et al., 2008).

Mitochondrial deficits

Parkin mutations *in vivo* are known to result in mitochondrial deficits in both humans and mice (Muftuoglu et al., 2004, Palacino et al., 2004). Mitochondria are a known source of H_2O_2, which causes oxidative damage when it interacts with cellular structures (Bao et al., 2009). Mitochondria produce H_2O_2 during normal respiration, however, H_2O_2 production can increase significantly when mitochondria become depolarized. Furthermore, *Parkin*, *PINK1*,

and *DJ-1* have all been reported to localize to mitochondria under conditions of stress. In the case of PD, levels of H_2O_2 may overwhelm cellular oxidative defenses following the loss of genes (*PINK1* and *Parkin*) that regulate mitochondria. This indicates that mitochondria and the reactive oxygen species they produce likely play an important role in the development of PD and that PD-causing genes play a role in protecting the cell from damage due to mitochondrial dysfunction (Dodson and Guo, 2007). Finally, both mitochondrial deficits and *Parkin* deficiencies have been linked to protein aggregation, which has also been implicated in PD (Dodson and Guo, 2007).

Role of Antioxidants

Genes involved in PD, specifically *DJ-1*, are thought to act as antioxidants, and loss of these genes is likely to result in increased levels of reactive oxygen species. In fact, DJ-1 localizes to mitochondria under conditions of stress, putting this protein in the correct location to clean up the increased H_2O_2 that is produced by dysfunctional mitochondria (Canet-Aviles et al., 2004, Zhang et al., 2005). Additionally, levels of known antioxidant proteins such as glutathione peroxidase are altered in PD. In the brains of deceased PD patients, *Gpx1* levels are lower in comparison to control brains and brains of patients with non-parkinsonian neurodegenerative diseases (Kish et al., 1985, Sian et al., 1994). Moreover, an increase in protein carbonyls is observed in PD patients, but not in the brains of Lewy body disease patients, which further suggests that oxidative damage is an important factor in the development of PD (Alam et al., 1997). Furthermore, levels of glutathione (GSH) and activity levels of *Gpx1* are decreased in the brains of patients (Kish et al., 1985, Zeevalk et

al., 2008), suggesting that the antioxidant system may be overworked and overwhelmed in PD. Several studies examining the levels of antioxidant activity in brain over the lifespan of rodents found changes in levels of glutathione or glutathione reductase specifically in the SN (Benzi et al., 1989, Chinta et al., 2007). Cells isolated from aged mice clear H_2O_2 more slowly than cells from young mice (Liddell et al., 2010), indicating a potential decrease in brain antioxidant capacity in the aging brain.

A large amount of related evidence suggests that levels of Gpx1 protein and activity are involved in protecting the brain against neurotoxic insults capable of resulting in PD. For instance, *Gpx1* knockout mice have decreased ability to cope with oxidative stressors despite not exhibiting a phenotype prior to being stressed. Although these mice have decreased body weight compared to wild type mice, they otherwise develop normally (Ho et al., 1997, Esposito et al., 1999). In contrast to these observations, de Haan et al found that *Gpx1*$^{-/-}$ mice are more susceptible to paraquat and H_2O_2 injections compared to wild type mice (de Haan et al., 1998). *Gpx1*$^{-/-}$ mice have an increase in secondary necrosis responses, indicating a decrease in tolerance to oxidative stress (Bajt et al., 2002). Aged mice lacking either *DJ-1* or *Parkin* have increased mitochondrial Gpx1 activity (Andres-Mateos et al., 2007, Rodriguez-Navarro et al., 2007), which may explain the lack of a parkinsonian phenotype in these mice as Gpx1 activity may compensate for the loss of neuroprotection by *Parkin* and *DJ-1*.

The purpose of my research has been to develop a progressive mouse model of PD by combining deficiency for a major antioxidant protein, Gpx1, with deficiencies for two proteins whose absence has been causally linked to the development of PD. I hypothesized

that the antioxidant deficiency would provide a chronic, low level of oxidative stress, that in combination with the *Parkin* and *DJ-1* deficiencies, would result in nigral neuron loss and decreased striatal dopamine, allowing for a better characterization of the mechanisms behind the originiation of the disease.

CHAPTER THREE
Methodology

Mice with mutations in genes that cause Parkinson's disease in humans were bred to create mice mutant for multiple parkinsonian genes. These mice were tested for a variety of phenotypes related to Parkinson's disease, including behavioral, neurochemical, and histological phenotypes. Because PD is an age-related disorder, cohorts of mice were tested at different ages, between 6 and 18 months, allowing for the examination of phenotypes that develop over time.

Animals

Parkin knockout mice and *DJ-1* knockout mice were generated as previously described (Goldberg et al., 2003, Goldberg et al., 2005) and backcrossed to strain C57BL/6J for 10 generations, then intercrossed for two generations to obtain homozygous double knockout mice (*Parkin*$^{-/-}$*DJ-1*$^{-/-}$) and wild-type controls. *Gpx1* knockout mice on a C57BL/6 background were obtained from Dr. Holly Van Remmen at The University of Texas Health Science Center at San Antonio. *Gpx1* knockout mice were crossed with *Parkin*$^{-/-}$*DJ-1*$^{-/-}$ double knockout mice for two generations to produce *Parkin*$^{-/-}$*DJ-1*$^{-/-}$*Gpx1*$^{+/-}$ mice, which were intercrossed to produce homozygous triple knockout mice (*Parkin*$^{-/-}$*DJ-1*$^{-/-}$*Gpx1*$^{-/-}$) and *Parkin*$^{-/-}$*DJ-1*$^{-/-}$ mice. When possible, paired littermates were used as controls. Experimental

procedures involving the use of animals or animal tissue were performed in accordance with the NIH Guidelines for Animal Care and Use and approved by the Institutional Animal Care and Use Committee at The University of Texas Southwestern Medical Center. Animals were housed in a climate-controlled facility with ventilated cages and standard commercial lab diet. Behavioral tests were performed between 10 AM and 4 PM during the 6 AM to 6 PM light cycle.

Behavioral tests

Locomotor: To measure spontaneous locomotor activity, mice were placed individually in a clean cage within an infrared photobeam activity monitor (San Diego Instruments) and were allowed to move freely in the dark for 2 hours. The number of beam breaks was recorded in 5-minute bins as a measure of locomotor activity.

Rotarod: The rotarod task is commonly used to measure motor function, and impairment is considered evidence of motor disease (Crawley, 2008). To measure motor function, mice were placed on an accelerating rotarod (IITC Life Science Inc) and the speed of rotation was increased from 5 to 45 revolutions per minute (RPM) over 5 minutes. The latency to fall from the rotarod was recorded. Data were collected for 4 trials per day for 2 days. In addition to analysis of motor performance, data were also analyzed for evidence of learning by comparing initial rotarod performance to performance during the final trial.

Fixed-speed rotarod and distraction measurements: To delineate the role of motor and non-motor factors ("distraction") on rotarod performance, fully trained mice were tested on a rotarod with fixed rotation rates of 5, 10, 15, or 20 RPM for a maximum of 5 minutes. For each speed, the latency to fall from the rotarod was recorded for 4 trials per day for 2 days. Mice were videotaped during the last two trials and the first 30 seconds of each video were analyzed with a stopwatch for time not focused on the task, which was measured as time in which the mice were not facing forward. All mice included in the video analysis stayed on the rotarod for the full 30 seconds.

Pharmacological studies of rotarod behaviors: In order to test whether performance on the rotarod was affected by cognitive abilities, mice were administered one of three normally prescribed for attention deficit disorder (methylphenidate, yohimbine or guanfacine) prior to rotarod testing at concentrations of 0.5, 1, 2, 5 and 10 mg/kg of methylphenidate, 1mg/kg of yohimbine or 0.2 mg/kg of guanfacine. Rotarod testing was performed as described previously, over the course of two days on an accelerating rod.

Odor test: To measure olfactory function, mice were placed in a clean cage containing a dry cotton swab within reach, and given 15 minutes to acclimate to their surroundings. The dry cotton swab was replaced by a cotton swab dipped in water for 3 minutes, followed by a cotton swab dipped in vanilla and finally a cotton swab dipped in urine collected from unfamiliar mice. Mice were videotaped during all three trials and the total time spent investigating each cotton swab was recorded using a stopwatch.

Digigait: To measure differences in gait, such as foot placement and stride length, mice were trained to walk on a treadmill equipped with a camera and attached to a computer with analysis software from Mouse Specifics. Mice were filmed walking at a speed of 30 cm/sec for at least 5 steps and their gait was analyzed using the provided software.

Forced Swim Test: Mice were placed in a beaker (16.5 cm diameter) of water (21-25° C) to a depth of seven inches. The mice remained in the water for 6 minutes and were then removed and allowed to dry in a clean dry cage before returning to their home cage. The water was changed between each subject. The mice were monitored from the side by camera and video images were taken for later analysis. The last four minutes of the test were scored for latency to the first immobility and total time spent immobile. The experimenter scoring the behavior was blind to the genotypes. Immobility was defined as no body or limb movement other than a minimal forelimb movement required for keeping the head above water.

Open Field Activity: Mice were placed in the periphery of a novel open field environment (44 cm x 44 cm, walls 30 cm high) and allowed to explore for five minutes. The animals were monitored from above by a video camera connected to a computer running video tracking software (Ethovision 3.0, Noldus, Leesburg, Virginia) to determine the time, distance moved and number of entries into two areas: the periphery (5 cm from the walls) and the center (14 cm x 14 cm). The open field arenas were wiped and allowed to dry between mice.

Elevated Plus Maze: Mice were placed in the center of a black Plexiglas elevated plus maze (each arm 30 cm long and 5 cm wide with two opposite arms closed by 25 cm high walls) elevated 31 cm and allowed to explore for five minutes. The animals were monitored from above by a video camera connected to a computer running video tracking software (Ethovision 3.0, Noldus, Leesburg, Virginia) to determine time spent in the open and closed arms, time spent in the middle, and the number of entries into the open and closed arm. The apparatus was wiped and allowed to dry between mice.

Dark-Light Activity: Mice were placed into a black Plexiglas chamber (25 cm x 26 cm) and allowed to explore for two minutes. After the habituation period, a small door was opened allowing them to access the light side of the apparatus (25 cm x 26 cm lit to approximately 1700 lux) for ten minutes. The animals were monitored by seven photobeams in the dark compartment and eight photobeams on the light side connected to a computer which recorded the time spent in each compartment, latency to enter the light side and the number of entrances to each compartment (Med-PC IV, Med Associates, St. Albans, VT). The dark-light apparatus was wiped and allowed to dry between mice.

Acoustic Startle Response: Acoustic startle response behavior was measured using a San Diego Instruments SR-Lab Startle Response System (San Diego, CA). Mice were placed into the Plexiglas holders and allowed to acclimate to the chamber and background white noise (70 dB) for five minutes. After acclimation, startle stimuli (80, 90, 100, 100 and 120 dB, 40

ms, white noise) were presented with an average interstimulus interval of 20 seconds (range 13 - 27 seconds). The Plexiglas holders were wiped and allowed to dry between mice.

Stereology of Dopaminergic Neurons

Stereology was performed according to previously described methods (Frank-Cannon et al., 2008). Brains were removed and placed in 10% neutral buffered formalin at 4° overnight, processed for paraffin embedding and sectioned in the coronal plane at 20-micron thickness. Every fifth slide was stained for unbiased stereology. Slides were deparaffinized, rehydrated in graded ethanol solutions and blocked with 5% normal goat serum for 1 hour prior to incubation in primary antibody (anti-tyrosine hydroxylase AB152, Chemicon) diluted 1:1000 at 4°C overnight. Sections were washed, incubated with biotinylated goat anti-rabbit secondary antibody, horseradish peroxidase conjugated avidin (ABC Elite, Vector) and were developed in 3,3'-Diaminobenzidine **(**DAB) solution with NiCl enhancement prior to dehydration and coverslipping. A microscope with a motorized stage and Stereoinvestigator software was used to count the tyrosine hydroxylase-positive neurons in the SN of each section, with a counting frame of 50 microns by 50 microns and a grid size of 100 microns by 100 microns. Bilateral total neuron numbers are reported.

Immunohistochemistry analysis of GFAP, DJ-1 Reactivity, Alpha-Synuclein and ubiquitin Inclusions

Brains were prepared using the same methods described above for immunohistochemistry of TH neurons, above. Primary antibodies used for GFAP (DAKO, Z0334) and *DJ-1*

(Neuromics, Park 7 Rb polyclonal) analysis were diluted to 1:1000 and 1:10,000, respectively, and staining was completed according to the TH immunohistochemistry protocol. Alpha-synuclein (Cell Signalling Syn205) and ubiquitin (Pierce PA5-17067) staining were completed using a mouse monoclonal immunohistochemistry kit (Abcam, 127055).

Stereology of Hippocampal Serotonergic Fibers

Mice were perfused with 50mL of 1x phosphate buffered saline (PBS) followed by 4% paraformaldehyde in 1xPBS. Following perfusion, the brain was removed and placed in 4% paraformaldehyde solution followed by 10% and 30% sucrose, each overnight. Brains were frozen in OCT and sectioned sagital at 14-micron thickness on a cryostat. Every 40th section was stained and used for unbiased stereology. Tissue was permeabilized with 0.3% Triton-X 100 prior to blocking with 10% normal donkey serum. Slides were then incubated in goat anti-serotonin transporter HTT-G0-af970 primary antibody (Frontier Labs) diluted 1:250 at 4°C overnight. Slides were washed in 1% fish skin gelatin prior to incubation in secondary antibody (donkey anti-goat Alexafluor 488, Jackson Immunoresearch 705-545-003) diluted 1:200. Slides were stained with 4',6-diamidino-2-phenylindole (DAPI) prior to coverslipping. Stereology was performed to measure immunoreactive fibers using the Spaceballs probe in Stereoinvestigator software, with a 200 x 200 micron grid and a hemisphere with a 10-micron radius.

In order to measure SERT expression in the hippocampus by densitometry, using Image J, sagittal brain sections were processed as described above, but a horseradish peroxidase conjugated donkey-anti goat secondary antibody (Jackson Immunoresearch 705-065-003) was used in place of the Alexafluor 488 conjugated secondary. DAB and final tissue processing was performed according to the TH immunostaining described above.

Measurement of tissue neurotransmitter levels

Mice were euthanized and the striatum was quickly dissected on an ice-cold glass dish, weighed, frozen on dry ice and stored at -80°C prior to analysis. Samples were combined with 50-fold (weight:volume) ice-cold 0.1 N perchloric acid containing 0.2 mM sodium metabisulfite. The tissue was disrupted by brief sonication and centrifuged at 4°C for 20 minutes at 15,000 x *g* to pellet proteins and cell debris. 200 µL of the supernatant was transferred to a clean tube, and 20 µL was injected onto an HPLC with a C18 column and eluted with isocratic MDTM mobile phase (ESA) at a rate of 0.6 mL/min. Monoamines were detected with a model 5014B electrochemical cell (ESA) set to a potential of +220 mV. Peak areas were normalized to tissue weight and compared to external standards for quantification.

Antioxidant Levels

Levels of antioxidants present in mouse brain were measured using a colorimetric assay provided by National Diagnostics. Brains were prepared by sonicating in artifical cerebrospinal fluid, on ice, spun at 10000 x *g* at 4°C. Supernatant was removed and allowed

to react with Fe^{2+} and a solution of xylenol orange overnight. Samples were normalized to a catalase (no H_2O_2) control. Absorbance was measured the following day.

Western Analysis and Oxyblots

Brains were dissected from 6-, 12-, and 18-month mice euthanized according to IACUC approved protocols by carbon dioxide asphyxiation and homogenized in 500 µL of Tris-based buffer containing EDTA, SDS and 1x protease inhibitor (Roche). For western blot, samples were boiled in 2x or 4x Laemmli buffer for 10 minutes prior to running on an SDS-PAGE (Biorad 5-10% or 12%). Following transfer to a nitrocellulose membrane, samples were tested for expression levels of antioxidant proteins. Antibodies used to detect proteins were: Gpx1 (Genetex, GTX116040S), SOD1 (Calbiochem, 574597), SOD2 (Upstate, 06-984), and TH (Chemicon, AB152). Oxyblot samples were reacted with 2,4-Dinitrophenylhydrazine (DNPH), which reacts with carbonyl groups, tagging them with 2,4-Dinitrophenol (DNP), using the Millipore system (S7150), prior to being loaded on an SDS-PAGE gel. Rabbit anti-DNP (Millipore, 90451) antibody was used to detect DNP-tagged protein. Otherwise, the methods used for the oxyblot were identical to the methods used in western blot analysis.

Reverse Transcriptase Quantitative PCR

Hippocampus and striatum were dissected from the brains of 12-month old mice euthanized according to IACUC approved protocols by carbon dioxide asphyxiation. Tissue was homogenized in 500 µL of Tri Reagent followed by shaking homogenate in 50 µL BCP and

extraction with isopropanol. Finally, samples were washed with 75% ethanol prior to use. cDNA was synthesized using Invitrogen Superscript (catalog #18080-051) oligoDT preparation. Quantitative analysis of reverse transcriptase PCR was performed on a 7500 Real Time PCR System (Applied Biosystems) using SYBR green master mix (Biorad #172-5120). Primers used were: TH (forward: 5'ttggctgaccgcacattt and reverse: 5'gccccccagagatgcaagt), tryptophan hydroxylase (TpH) (forward: 5'gggctggtgaaagcacttaga and reverse: 5'tggattcgatatgaagcatgttg), SERT (forward: cccgcagagctctcagtctt and reverse: agctcttggttcttggttttgaa), DAT (forward: 5'ggagtgctcattgaagccatt and reverse: 5'catctgcttgatgtcatcactga), cyclophilin (forward: 5`tggagagcaccaagacagaca and reverse: 5`tgccggagtcgacaatgat).

Statistics

Data analysis was performed using SigmaPlot software. One-way ANOVA was used to analyze the differences between multiple genotypes. Where appropriate, repeated measures two-way ANOVA was used to account for multiple trials. $p<0.05$ was considered to be significant and Tukey's post hoc analysis was used to further analyze differences between groups. Planned comparisons were performed using Student's t-test.

CHAPTER FOUR

RESULTS

Mice deficient for combinations of genes that have been causally linked to PD were examined for signs of oxidative stress and damage, typical PD pathology, and behaviors related to PD.

Results

Double and Triple Mutant Mice Appear Normal

All mutant phenotypes, *Parkin*$^{-/-}$, *DJ-1*$^{-/-}$, *Gpx1*$^{-/-}$, *Parkin*$^{-/-}$*DJ-1*$^{-/-}$ and *Parkin*$^{-/-}$*DJ-1*$^{-/-}$*Gpx1*$^{-/-}$ appear grossly phenotypically normal compared to wild type mice. There are no obvious motor deficits and mice appear to function normally and do not appear to have health problems. The only phenotypic difference observed between genotypes is weight (Figure 4-1) where mutant mice are generally smaller than wild type, beginning between ages 8-12

months ($p < 0.0001$, two-way ANOVA).

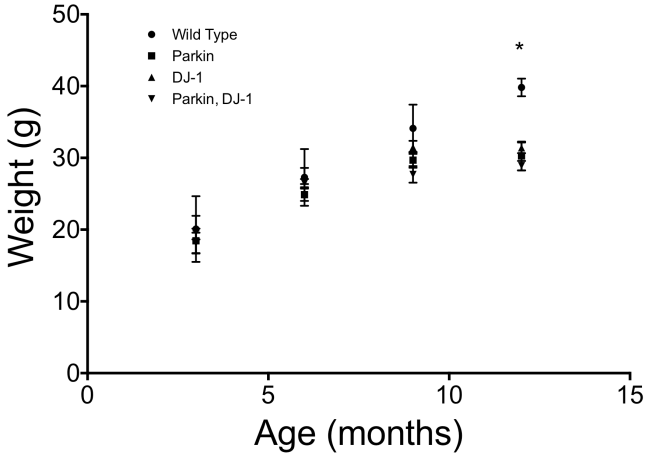

Figure 4-1. Wild type mice weigh significantly more than *Parkin*$^{-/-}$, *DJ-1*$^{-/-}$ and *Parkin*$^{-/-}$*DJ-1*$^{-/-}$ beginning at 12 months of age. Separate groups of mice were weighed at 3, 6, 9 and 12 months. All mice gain weight throughout their lifetimes, although the mutant mice appear to plateau about 12 months. No differences were observed between any mutant genotypes. Bars represent mean ± SEM weight in grams. ($p < 0.0001$, two way ANOVA).

No changes in hydrogen peroxide levels, levels of carbonyl-tagged proteins or brain antioxidant levels

Hydrogen peroxide levels in the brains of mice deficient for *Parkin, DJ-1* and *Gpx1* were measured using a colorimetric assay for an expected increase in H_2O_2 levels in mice deficient for *Gpx1* (Figure 4-2). Contrary to this hypothesis, there were no differences in H_2O_2 levels in any of the genotypes tested, compared to wild type ($p=0.877$, one way ANOVA).

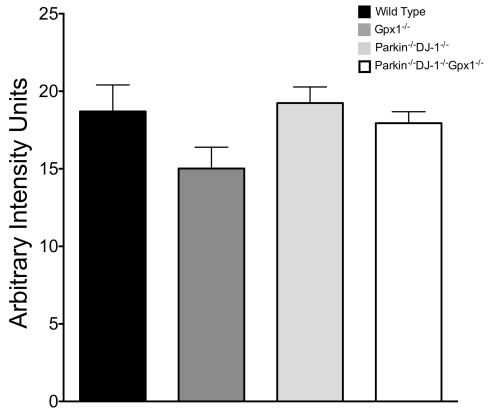

Figure 4-2. Hydrogen peroxide levels were unchanged in PD mutant mice compared to wild type. Hydrogen peroxide levels in whole brain samples of mice were measured by colorimetric assay from National Diagnostics and normalized to total protein levels and to a catalase no hydrogen peroxide control. Bars represent mean ± SEM arbitrary intensity units at 560 nm ($p = 0.15$)

Additionally, I examined the levels of carbonyl-tagged proteins by oxyblot, as a measure of oxidative damage to proteins in wild type, *Parkin*$^{-/-}$*DJ-1*$^{-/-}$ and *Parkin*$^{-/-}$*DJ-1*$^{-/-}$*Gpx1*$^{-/-}$ mice. Following immunoblotting against the DNP-tagged protein, exposure levels were quantified using ImageJ image analysis software (Figure 4-3). No differences were found between any of the genotypes tested ($p \geq 0.4$, one way ANOVA).

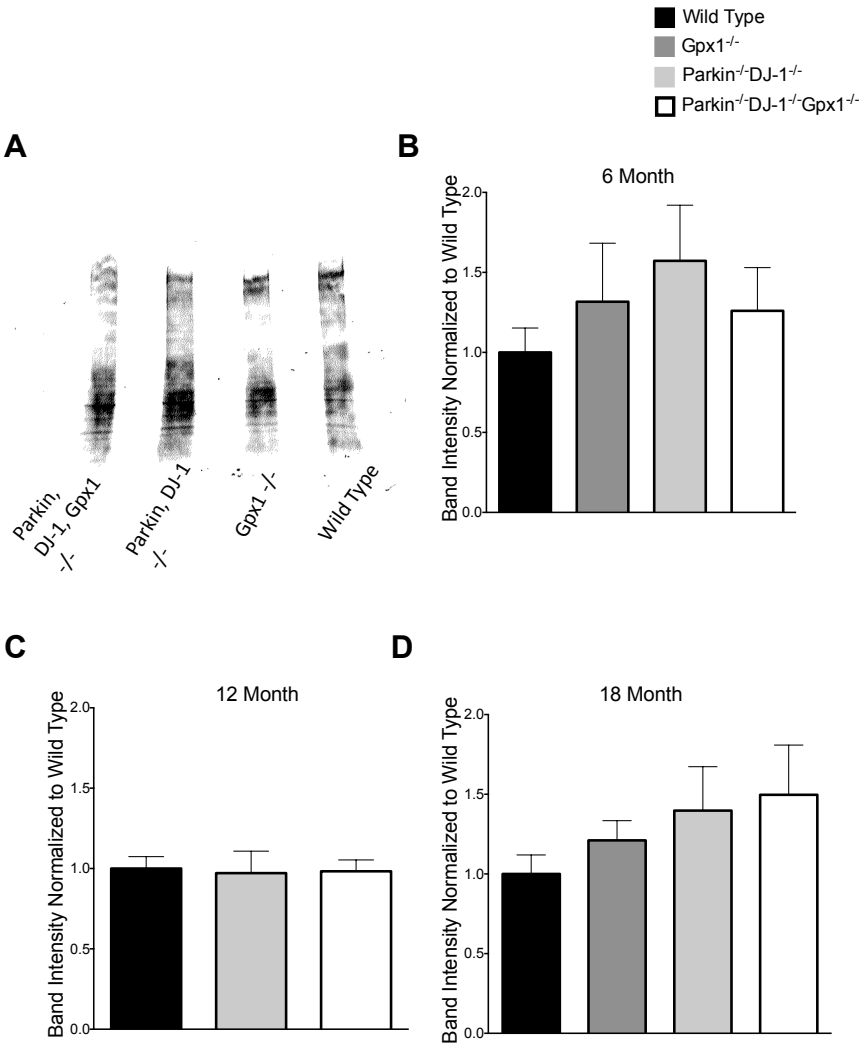

Figure 4-3. Levels of oxidized proteins were unchanged in PD mutant mice compared to wild type. Whole-brain samples from mice were reacted with DNPH in order to tag carbonyl groups with DNP; western blotting of DNP-tagged proteins (A) was performed at 6 (B), 12 (C) and 18 (D) months. All genotypes have similar signal intensities of DNP-tagged proteins. Bars represent mean ± SEM band intensity normalized to wild type ($p \geq 0.4$)

Finally, I measured the levels of major brain antioxidants Gpx1, SOD1 and SOD2 in the brains of wild type, *Gpx1*$^{-/-}$, *Parkin*$^{-/-}$*DJ-1*$^{-/-}$ and *Parkin*$^{-/-}$*DJ-1*$^{-/-}$*Gpx1*$^{-/-}$ mice by immunoblot. Although the analysis determined that there was the expected loss of Gpx1 in the mice

deficient for this protein, no other changes in levels of antioxidant proteins was observed, as can be seen in the representative images of SOD1 and SOD2 (Figure 4-4).

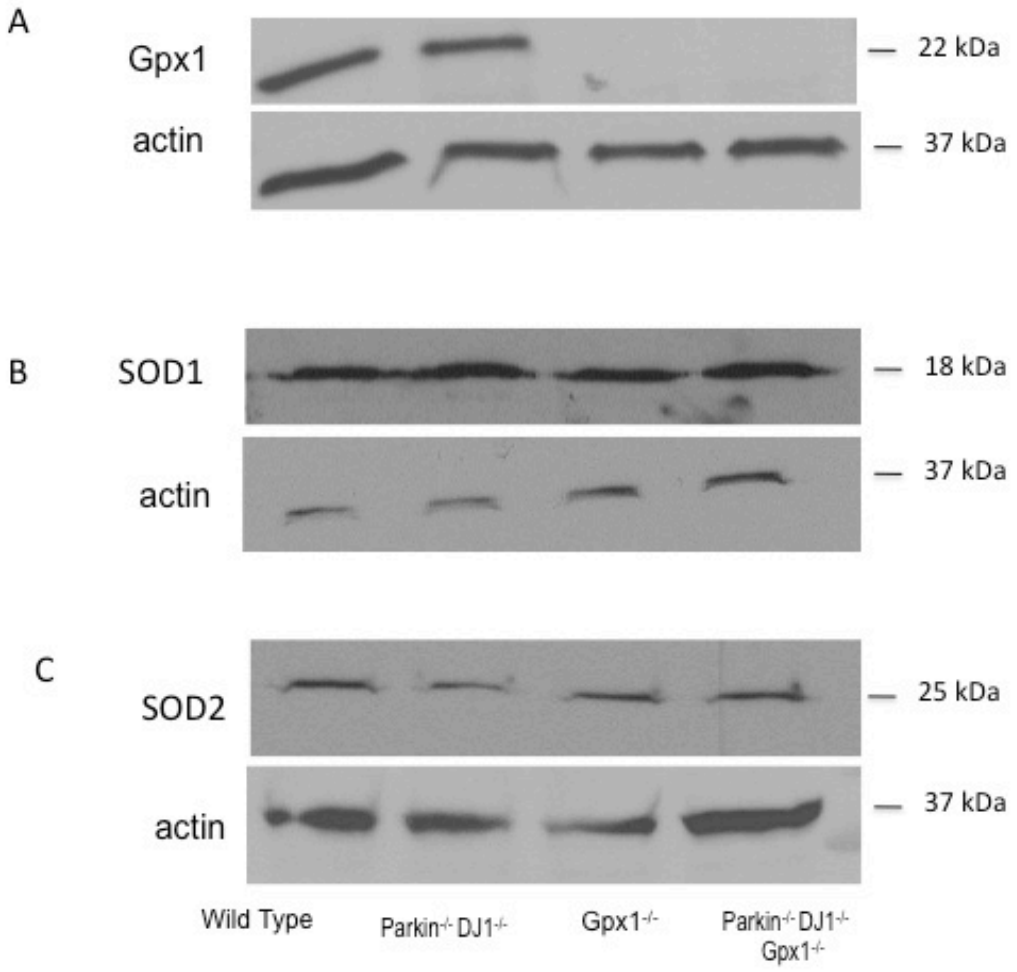

Figure 4-4. Levels of antioxidant proteins were unchanged in PD mutant mice compared to wild type. Protein levels of glutathione peroxidase 1 (A), supderoxide dismutase 1 (B) and superoxide dismutase 2 (C) were measured by western blot at 6, 12 and 18 months of age. Representative blots from 18 month mice are shown here. No differences were observed between any of the genotypes at any age.

Mutant mice do not exhibit PD-related pathology

I examined the brain pathology of wild type, *Gpx1*$^{-/-}$, *Parkin*$^{-/-}$*DJ-1*$^{-/-}$ and *Parkin*$^{-/-}$*DJ-1*$^{-/-}$ *Gpx1*$^{-/-}$ mice at 18 months, a time point at which the mice are considered aged and where I would expect to see the greatest pathological changes. I stained brain slices for GFAP, ubiquitin and alpha-synuclein expression. GFAP is a marker of reactive astrogliosis, and increased GFAP immunostaining is considered to be a marker of damage to the brain. Ubiquitin and alpha-synuclein have both been found to form inclusions in the brains of Parkinson's disease patients. I examined the brains for signs of ubiquitin or synuclein inclusions, considered to be markers of parkinsonian pathology in these mouse models. However, compared to wild type, all other genotypes appeared phenotypically normal with no reactive astrocytes or ubiquitin or synuclein immunoreactive inclusions (Figure 4-5).

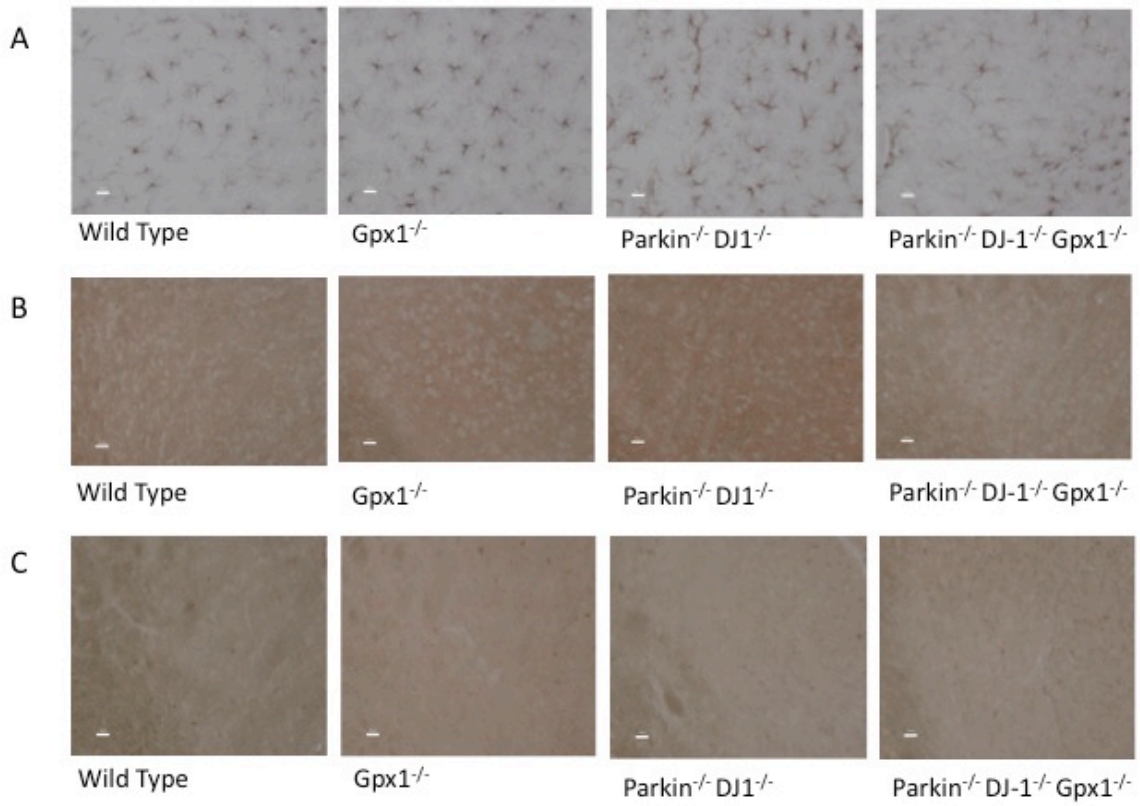

Figure 4-5. Mutant mice have phenotypically normal brains. Coronal brain sections from wild type, *Gpx1*$^{-/-}$, *Parkin*$^{-/-}$*DJ-1*$^{-/-}$ and *Parkin*$^{-/-}$*DJ-1*$^{-/-}$*Gpx1*$^{-/-}$ 18 month old mice were stained for GFAP (A), synuclein (B) or ubiquitin staining (C). No differences were observed between any genotypes.

Parkin$^{-/-}$DJ-1$^{-/-}$Gpx1$^{-/-}$ mice have normal numbers of nigral neurons

Triple knockout mice bearing combined loss-of-function mutations in the PD-linked genes *Parkin* and *DJ-1*, as well as the antioxidant *Gpx1* gene, were born at the expected Mendelian ratio and had no apparent differences in viability or longevity compared to wild-type mice. Because age-dependent loss of dopaminergic neurons in the SN is the primary pathological characteristic of PD and the cause of the motor symptoms observed in patients, I investigated whether *Parkin$^{-/-}$DJ-1$^{-/-}$Gpx1$^{-/-}$* mice exhibit progressive loss of nigral neurons. Coronal brain sections were stained using an antibody specific for TH, a marker of dopamine-containing neurons, and rigorous stereological methods were used to estimate the number of dopaminergic neurons in the SN of mice at ages 6, 12 and 18 months. I observed statistically similar numbers of TH-immunoreactive neurons in each genotype at age 6 months (Figure 4-6B), 12 months (Figure 4-6C) and 18 months (Figure 4-6D). These data indicate that the number of dopaminergic neurons is not significantly altered in *Parkin$^{-/-}$DJ-1$^{-/-}$Gpx1$^{-/-}$* mice.

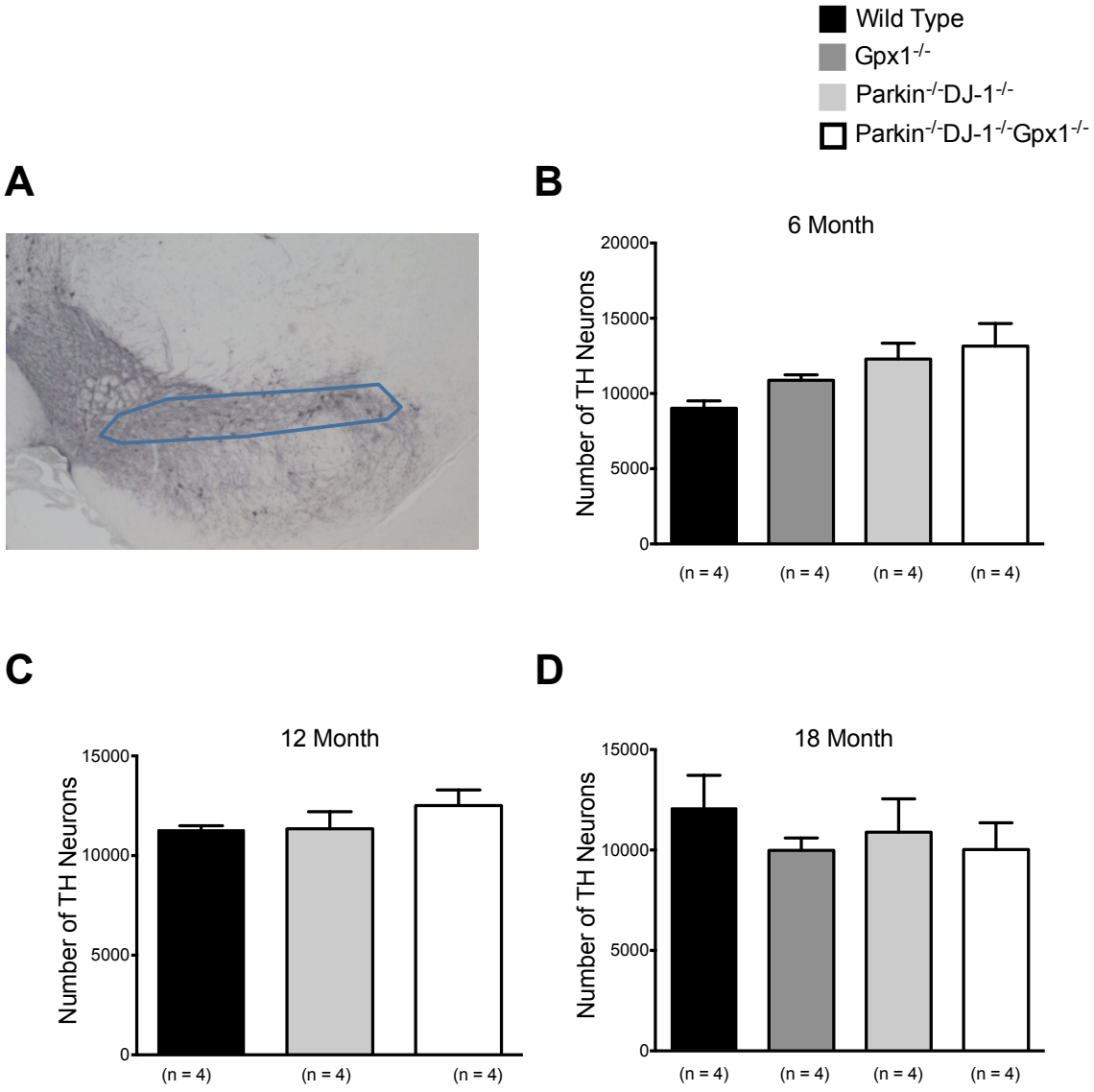

Figure 4-6. *Parkin*$^{-/-}$*DJ-1*$^{-/-}$ and *Parkin*$^{-/-}$*DJ-1*$^{-/-}$*Gpx1*$^{-/-}$ mice have normal substantia nigra cell numbers. The number of dopaminergic tyrosine hydroxylase (TH)-positive neurons in the substantia nigra estimated by unbiased stereology. Nigral tyrosine hydroxylase staining, with substantia nigra indicated in blue. Separate cohorts of mice were analyzed at ages 6 (B), 12 (C) and 18 (D) months, n=4-6 mice per genotype at each age. Bars show the mean ± SEM estimated total number of TH-positive neurons. Means were not significantly different at any age. ($p > 0.05$)

Increased striatal dopamine in Parkin$^{-/-}$*DJ-1*$^{-/-}$*Gpx1*$^{-/-}$ *mice*

Nigral dopaminergic neurons project to the dorsal striatum (caudate and putamen) and compensatory changes in dopamine levels and dopamine turnover at presynaptic terminals in the striatum have been hypothesized to occur during presymptomatic stages of PD (Bernheimer et al., 1973). I therefore investigated whether *Parkin*$^{-/-}$*DJ-1*$^{-/-}$*Gpx1*$^{-/-}$ mice have altered levels of striatal dopamine even with normal nigral neuron numbers. I used high performance liquid chromatography (HPLC) with electrochemical detection to measure the levels of dopamine and its metabolites 3,4-Dihydroxyphenylacetic acid (DOPAC), homovanillic acid (HVA) and 3-methoxytyramine (3-MT) in the striatum of mice at ages 6, 12 and 18 months. *Parkin*$^{-/-}$*DJ-1*$^{-/-}$ mice and mice with a single *Gpx1* deficiency showed no change in striatal dopamine levels compared to wild type mice (Figure 4-7). Surprisingly, dopamine levels were significantly elevated in *Parkin*$^{-/-}$*DJ-1*$^{-/-}$*Gpx1*$^{-/-}$ mice at 18 months ($p<0.05$, one-way ANOVA). At earlier ages, striatal dopamine levels are not significantly different by ANOVA, however, t-test shows significant differences between wild-type and *Parkin*$^{-/-}$*DJ-1*$^{-/-}$*Gpx1*$^{-/-}$ mice at age 12 months (p= 0.0019) and 6 months (p=0.0475) (Figure 4-7). These results suggest that *Parkin*$^{-/-}$*DJ-1*$^{-/-}$*Gpx1*$^{-/-}$ mice have compensatory changes in striatal dopamine levels.

I found no significant differences in dopamine turnover, calculated as the ratio of dopamine metabolites to dopamine (Figure 4-8).

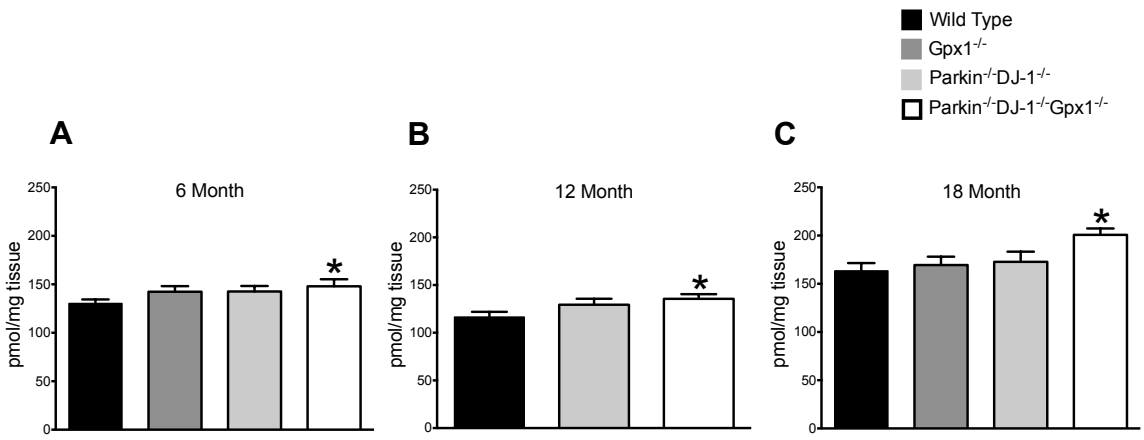

Figure 4-7. Striatal dopamine is increased in *Parkin*$^{-/-}$*DJ-1*$^{-/-}$*Gpx1*$^{-/-}$ mice. Levels of striatal dopamine (DA) measured by HPLC with electrochemical detection. Separate cohorts of mice were analyzed at ages 6 (A), 12 (B) and 18 (C) months, n=6-11 mice per genotype at each age. Bars show the mean ± SEM of the level of dopamine measured from microdissected striatum. Asterisks indicate significant differences compared to wild-type mice at the same age (*$p<0.05$, t-test). *Parkin*$^{-/-}$*DJ-1*$^{-/-}$*Gpx1*$^{-/-}$ mice have increased DA levels compared to wild type (**$p<0.01$, one-way ANOVA, Tukey's post-hoc) at age 18 months but not at 6 or 12 months.

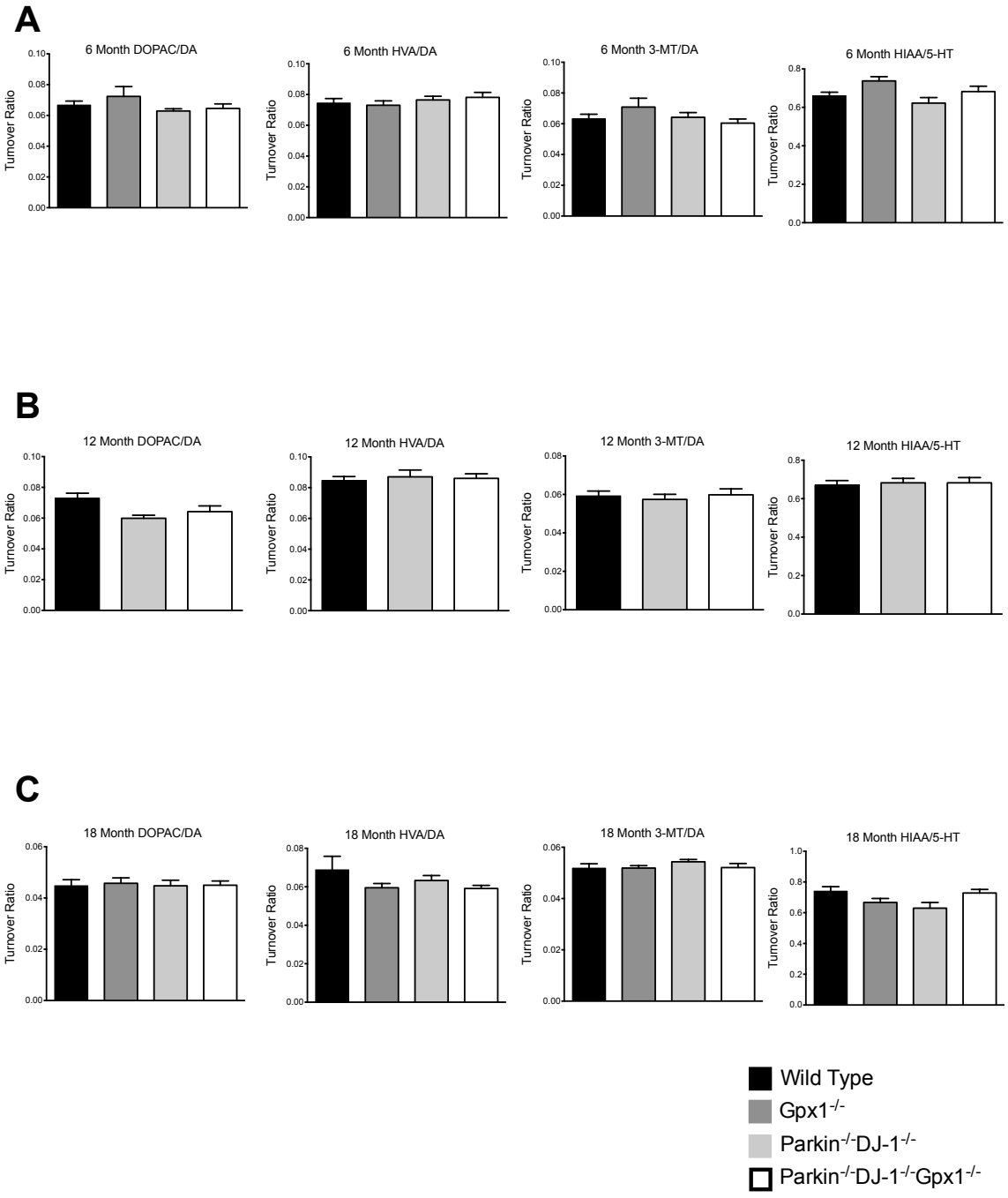

Figure 4-8. Turnover rates of dopamine and serotonin were not altered in the striatum of *Gpx1*$^{-/-}$, *Parkin*$^{-/-}$*DJ-1*$^{-/-}$, or *Parkin*$^{-/-}$*DJ-1*$^{-/-}$*Gpx1*$^{-/-}$ mice compared to wild type at 6 (A), 12 (B) or 18 (C) months. Turnover of dopamine was calculated as the ration of DOPAC/DA (first column), HVA/DA (second column), 3-MT/DA (third column) and turnover of serotonin was calculated as HIAA/5-HT (fourth column). Bars represent ± SEM of the ratio of neurotransmitter and metabolite levels. ($p > 0.05$ by one way ANOVA)

Serotonin levels are altered in multiple brain regions of Parkin$^{-/-}$DJ-1$^{-/-}$ mice

In addition to showing an increase in striatal dopamine, our HPLC analysis revealed that serotonin levels are significantly increased in the striatum of *Parkin$^{-/-}$DJ-1$^{-/-}$* mice and *Parkin$^{-/-}$DJ-1$^{-/-}$Gpx1$^{-/-}$* mice at age 12 months (p<0.01, one-way ANOVA) and in the striatum of *Parkin$^{-/-}$DJ-1$^{-/-}$* mice at 18 months (p<0.05, one-way ANOVA) (Figure 4-9). No consistent differences were observed in serotonin turnover or in the levels of 5-hydroxyindoleacetic acid (5-HIAA), the primary metabolite of serotonin (Figure 4-8).

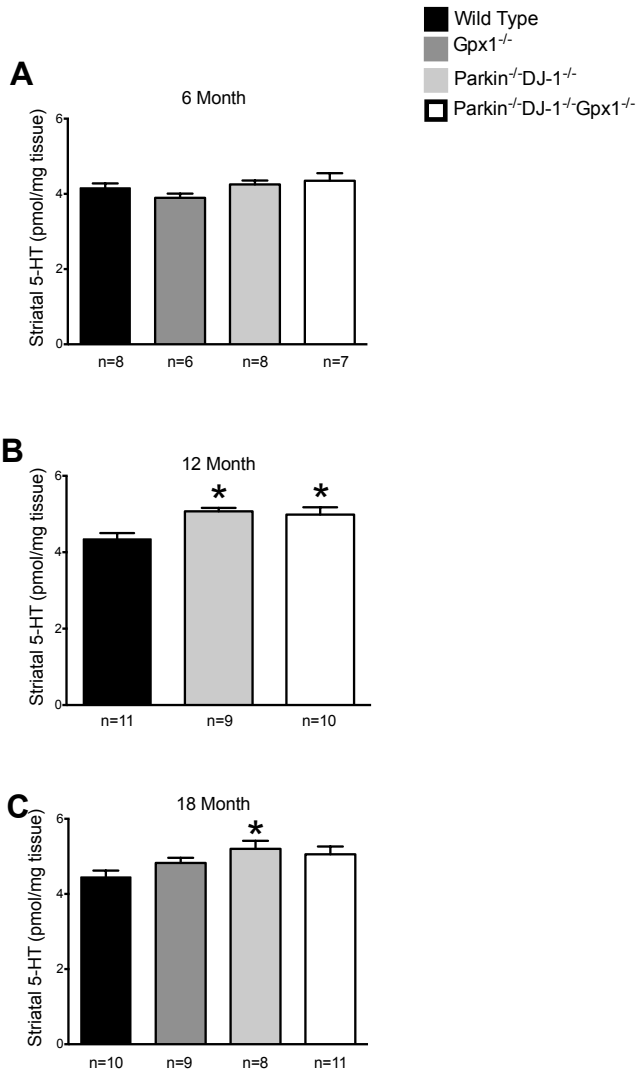

Figure 4-9. Striatal serotonin is increased in *Parkin$^{-/-}$DJ-1$^{-/-}$* and *Parkin$^{-/-}$DJ-1$^{-/-}$Gpx1$^{-/-}$* mice. Levels of striatal serotonin (5-HT) measured by HPLC with electrochemical detection. Separate cohorts of mice were analyzed at ages 6 (A), 12 (B) and 18 (C) months, n=6-11 mice per genotype at each age. Bars show the mean ± SEM of the level of serotonin measured from microdissected striatum. *p<0.05, one-way ANOVA compared to wildtype

I found no genotype-dependent differences in dopamine levels in other brain regions (data not shown). However, serotonin was significantly elevated in the hippocampus of *Parkin$^{-/-}$DJ-1$^{-/-}$* mice compared to wild type (p<0.05, one-way ANOVA) (Figure 4-10).

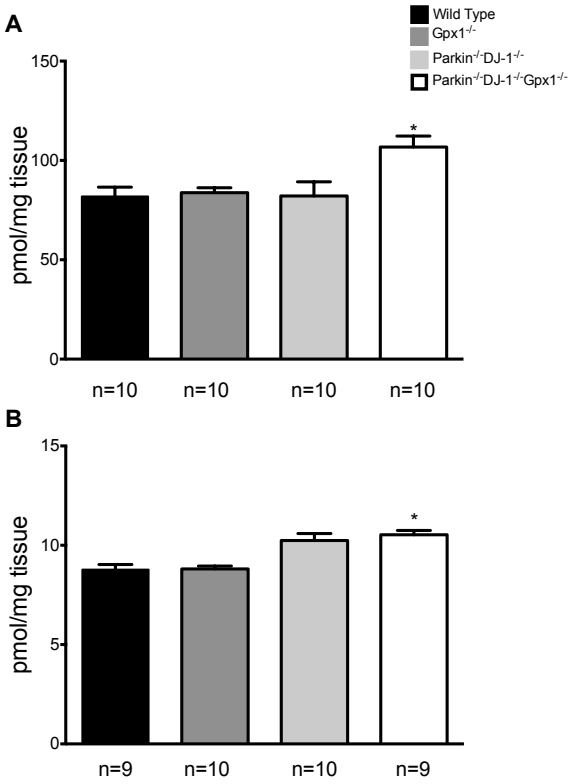

Figure 4-10. Serotonin levels are increased in the hippocampus and prefrontal cortex of 15- month *Parkin*$^{-/-}$*DJ-1*$^{-/-}$ mice, compared to wild type mice at the same age. (A) Levels of hippocampal serotonin (5-HT) and (B) levels of cortical 5-HT measured by HPLC with electrochemical detection. Bars show the mean ± SEM (n=10 per genotype). (*p<0.05, one-way ANOVA).

Hippocampal serotonergic fibers are unchanged in PD mutant mice

One possible cause of the observed increase in hippocampal serotonin levels is an increase in serotonergic projections to the hippocampus. Therefore, I quantified serotonin-releasing fibers, defined as axons immunoreactive for the serotonin transporter (SERT) in the hippocampus, by stereological analysis. The estimated summed length of all serotonergic fibers in the hippocampus was calculated (Figure 4-11). Although there was a trend towards increased SERT-positive fibers in the hippocampus of *DJ-1*$^{-/-}$ mice and *Parkin*$^{-/-}$*DJ-1*$^{-/-}$ mice compared to both wild type and *Parkin*$^{-/-}$ mice, this difference was not statistically significant (Figure 4-11B). Together, these data indicate the significant effects of *Parkin* and *DJ-1* deficiency on the regulation of non-catecholamine neurotransmitters in the brain.

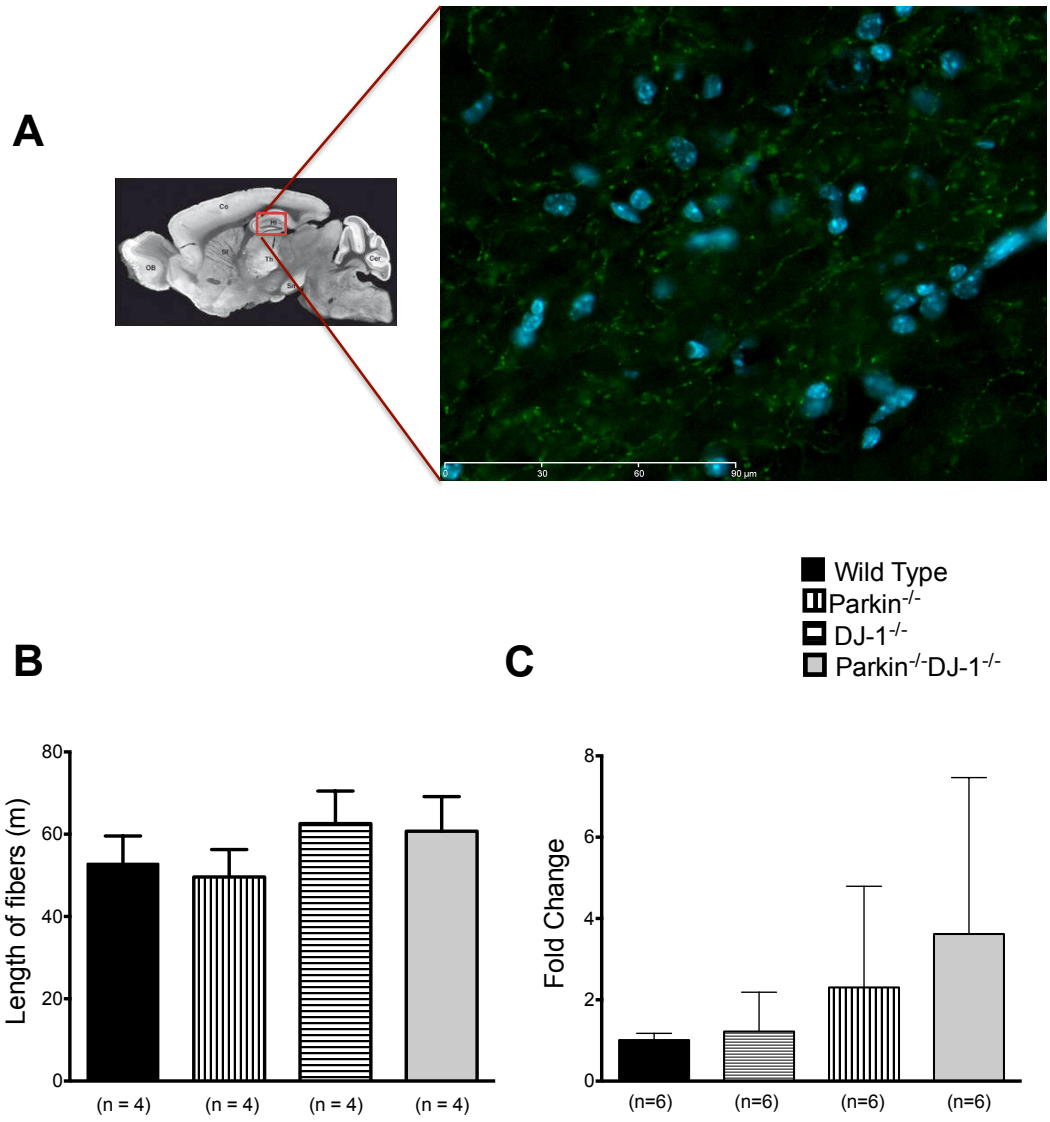

Figure 4-11. Increased serotonin levels may be due to increased production. (A) Sagittal brain sections were stained for SERT expression (green), with a DAPI counterstain (blue). (B) Length of serotonergic fibers in the hippocampus measured by unbiased stereology (n=4 mice per genotype). (C) Levels of TpH mRNA are unchanged in the hippocampus, although the *Parkin*$^{-/-}$*DJ-1*$^{-/-}$ mice have a trend toward increased TpH. Bars show the mean ± SEM of total length of fibers and fold change, respectively. (p = 0.1)

Levels of tryptophan hydroxylase mRNA are not increased in the hippocampus of PD mutant mice

One other potential explanation for the increase in neurotransmitter levels is an increase in neurotransmitter production. Therefore, I examined the hippocampal mRNA levels of TpH, the enzyme involved in the rate-limiting step of serotonin production, by quantitative reverse transcriptase polymerase chain reaction (PCR) in wild type, *Parkin*$^{-/-}$, *DJ-1*$^{-/-}$ and *Parkin*$^{-/-}$*DJ-1*$^{-/-}$ mice at 12 months, as a measure of enzyme levels and a proxy measure of how much serotonin is produced in the hippocampus. No differences were observed between the wild type and single knockout mice, and there were no statistical differences between any of the genotypes by one-way ANOVA ($p = 0.2$). Nevertheless, compared to wild type, *Parkin*$^{-/-}$*DJ-1*$^{-/-}$ mice have a trend toward a 3-fold increase in TpH levels (Figure 4-11C). However, this increase is not significant ($p = 0.1$, by Student's t-test). These data indicate that increased TpH does not account for the increase in serotonin levels. Nevertheless, the trend toward an increase in TpH suggests that increased production of serotonin may partially account for the observed increase of this neurotransmitter in the hippocampus.

Densitometry analysis of TH and SERT expression

In addition to the studies mentioned above, I also examined the expression levels of TH and SERT by densitometry analysis with Image J following immunohistochemical staining of tissue sections taken from mice. TH staining was examined in wild type, *Gpx1*$^{-/-}$, *Parkin*$^{-/-}$*DJ-1*$^{-/-}$ and *Parkin*$^{-/-}$*DJ-1*$^{-/-}$*Gpx1*$^{-/-}$ mice at 6, 12 and 18 months of age (Figure 4-12A, C). No differences were found in striatal TH expression between the genotypes at any age, or

between genotypes when all ages were combined ($p \geq 0.3$, one-way ANOVA). SERT staining was performed on wild type, *Parkin*$^{-/-}$, *DJ-1*$^{-/-}$ and *Parkin*$^{-/-}$*DJ-1*$^{-/-}$ mice at 12 months (Figure 4-12B, D). No differences were found between hippocampal SERT expression in any of the genotypes ($p = 0.9$, one-way ANOVA).

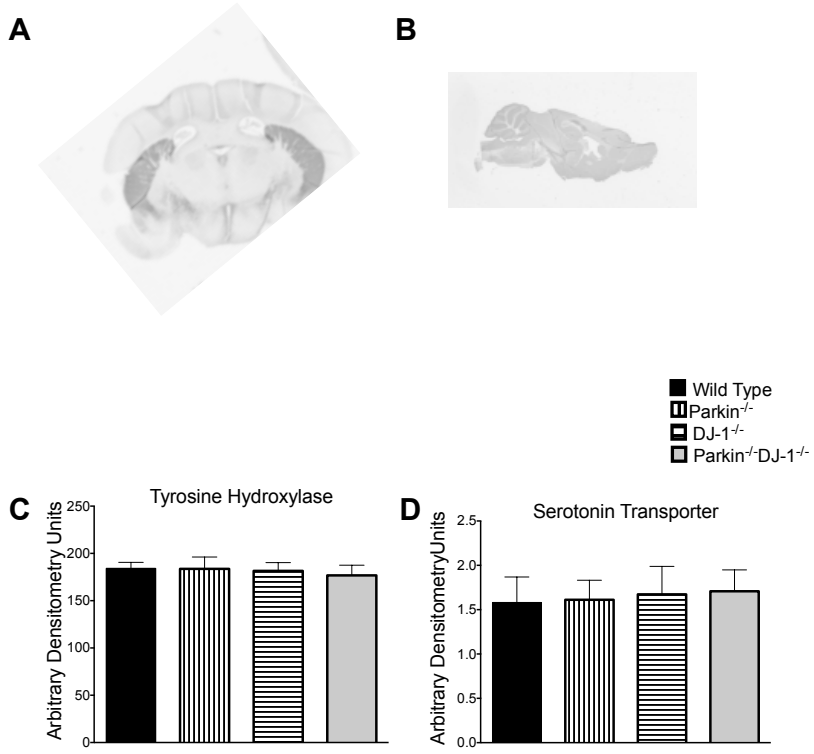

Figure 4-12. No differences in TH or SERT densitometry. Brain sections were stained for either TH (A) or SERT (B) expression. Densitometry measures were made using Image J. No differences were observed in the intensity of the staining for either TH (C) or SERT (D). Bars show the mean ± SEM of arbitrary units as measured by Image J. ($p \geq 0.3$)

Behavior

Because Parkinson's disease causes deficits in motor function, I investigated whether mice with combined PD-linked mutations have altered performance in established behavioral tests

of motor function. Furthermore, because age is the greatest risk factor for PD, separate cohorts of mice were tested at ages 6, 12, and 18 months to assess whether motor abilities changed with age.

Locomotor behavior of PD mutant mice was unchanged compared to wild type

Spontaneous locomotor behavior was measured by placing mice individually in automated activity monitors and tabulating the number of infrared beam breaks due to locomotor activity over the course of two hours. As expected, locomotor activity decreases during the two-hour test as the animals acclimate to a new environment (Figure 4-13). At all three ages tested, the activity of mutant mice was indistinguishable from wild type mice ($p = 0.2$, two way ANOVA).

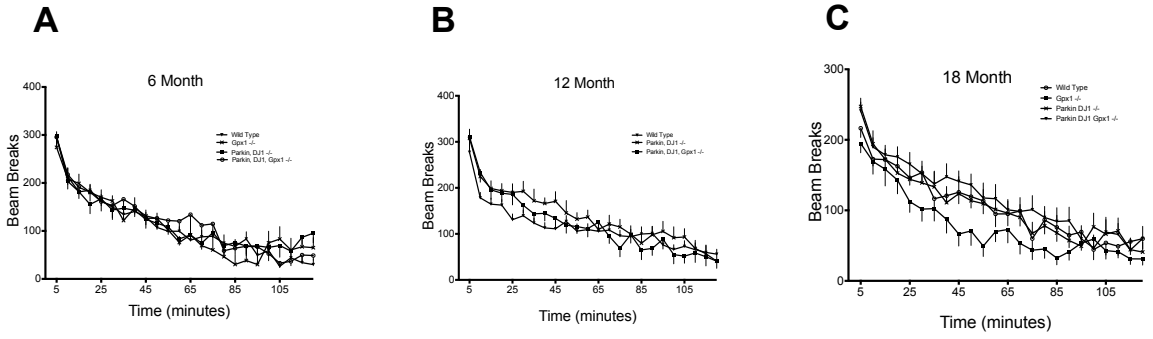

Figure 4-13. *Parkin*$^{-/-}$*DJ-1*$^{-/-}$ and *Parkin*$^{-/-}$*DJ-1*$^{-/-}$*Gpx1*$^{-/-}$ mice have normal locomotor activity. Spontaneous locomotor activity was measured for separate cohorts of mice at age 6 (A), 12 (B) and 18 months (C), n=6-8 mice per genotype, 8-11 mice per genotype and 9-11 mice per genotype, respectively. For all ages, the mice acclimated to the novel environment, but there are no significant differences between genotypes (one-way ANOVA). Symbols represent the mean ± SEM number of infrared beam breaks in each 5-minute period of the 2-hour test.

Parkinson's Disease mutant mice do not exhibit gait abnormalities

I examined gait differences in wild type, *Parkin*$^{-/-}$*DJ-1*$^{-/-}$, *Gpx1*$^{-/-}$, and *Parkin-/-DJ-1*$^{-/-}$*Gpx1*$^{-/-}$ mice at 12 and 18 months. MPTP-treated mice have been reported to have decreased stride length compared to untreated mice (Amende et al., 2005) and decreased paw angle (the angle of the hind foot to the body) has been reported in mouse models of arthritis (Vincelette et al., 2007). Here, stride length and paw angle were compared between genotypes at ages 12 and 18 months (Figure 4-14). Although both stride length and paw angle were decreased at 18 months compared to 12 months, possibly an indication of motor deficits due to age, I found no differences in either measure of gait dynamics between genotypes at either age ($p \geq 0.1$). Anecdotally, I observed that mutant mice were more willing to perform the task than wild type mice as I found it easier to collect the minimum video length of five steps for analysis in the triple mutant mice. It should be noted, however, that analysis was performed prior to being trained by a MouseSpecifics employee, and later training indicated that our methods had room for optimization. However, due to observations of the mice and results of other behavioral tests, I find it unlikely that making changes to optimize the experimental conditions would greatly change the outcome of this particular experiment.

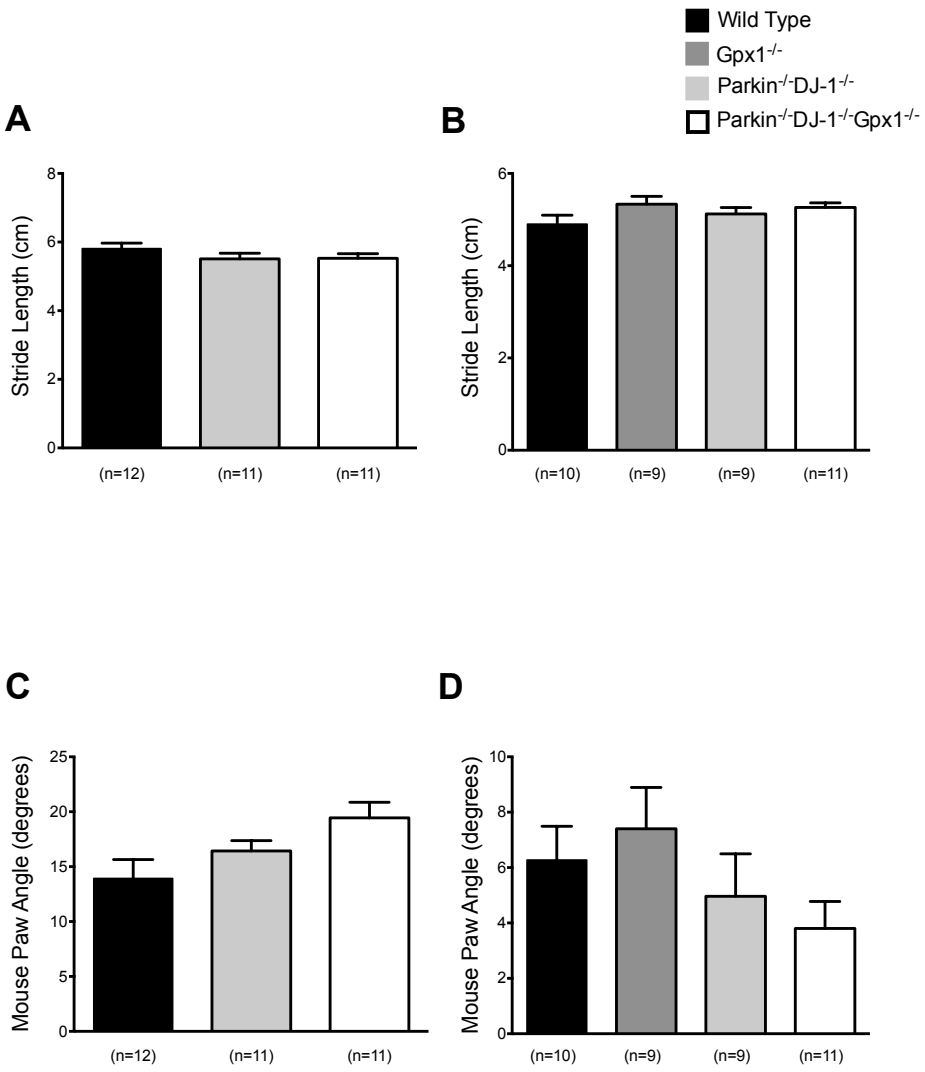

Figure 4-14. Comparison of stride length at 12 (A) and 18 (B) months, and paw angle at 12 (C) and 18 (D) months between genotypes by Digigait analysis showed no differences in the gait between genotypes. Bars represent mean ± SEM stride length (cm) and paw angle (degrees), respectively. ($p \geq 0.2$, $p \geq 0.1$, respectively by one way ANOVA)

Improved rotarod performance of Parkin$^{-/-}$DJ-1$^{-/-}$ mice compared to wild type

In addition to the locomotor test, I analyzed the behavior of mice on the rotarod test, which measures the ability of mice to stay on top of a rotating horizontal rod as the speed of rotation accelerates from 5 to 45 RPM over 5 minutes. The rotarod test has been used for many years to detect rodent neurological deficits affecting motor coordination and balance (Dunham and Miya, 1957). Separate cohorts of mice were tested at ages 6, 12 and 18 months and the latency to fall off the rotarod was analyzed by two-way repeated measures ANOVA with trial as the repeated measure. All genotypes showed increasing latency over the 8 trials, as they learned to perform the task. Contrary to our expectations, *Parkin$^{-/-}$DJ-1$^{-/-}$* mice and *Parkin$^{-/-}$DJ-1$^{-/-}$Gpx1$^{-/-}$* mice were able to stay on the rotarod significantly longer than wild-type mice. At age 6 months, there was a trend towards increased latency to fall in *Parkin$^{-/-}$DJ-1$^{-/-}$* and *Parkin$^{-/-}$DJ-1$^{-/-}$Gpx1$^{-/-}$* mice but this difference was not statistically significant (Figure 4-15A). However, at ages 12 and 18 months, *Parkin$^{-/-}$DJ-1$^{-/-}$* and *Parkin$^{-/-}$DJ-1$^{-/-}$Gpx1$^{-/-}$* mice showed increased latency to fall compared to wild type mice in trials 6, 7 and 8 ($p<0.05$ at 12 months and $p<0.001$ at 18 months, two-way repeated measures ANOVA) (Figure 4-15B, C). There was no significant difference between wild-type and *Gpx1$^{-/-}$* mice at any age nor was there an additive effect of *Gpx1$^{-/-}$* to *Parkin$^{-/-}$DJ-1$^{-/-}$*.

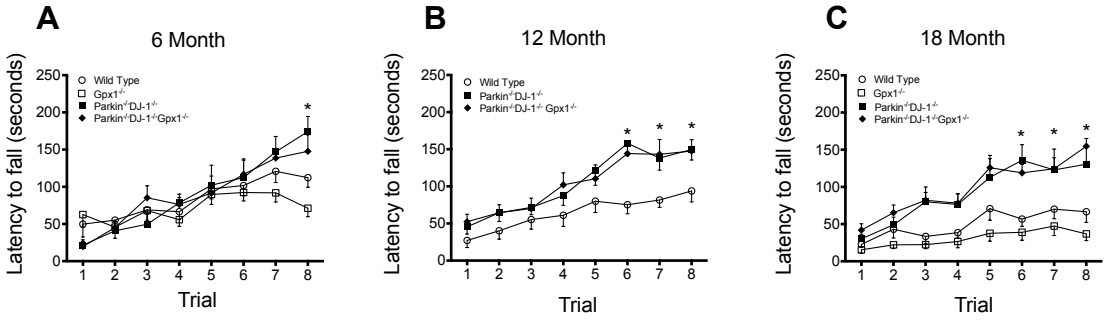

Figure 4-15. *Parkin*$^{-/-}$*DJ-1*$^{-/-}$ and *Parkin*$^{-/-}$*DJ-1*$^{-/-}$*Gpx1*$^{-/-}$ mice have improved rotarod performance compared to wild type. The latency to fall off an accelerating rotating rod was measured for separate cohorts of mice at age 6 (A), 12 (B) and 18 months (C), n=6-8 mice per genotype, 10-11 mice per genotype and 9-11 mice per genotype, respectively. Symbols represent the mean ± SEM time (seconds) before falling off the rod for each of 8 trials. While all genotypes learned the task over multiple trials, *Parkin*$^{-/-}$*DJ-1*$^{-/-}$ and *Parkin*$^{-/-}$*DJ-1*$^{-/-}$*Gpx1*$^{-/-}$ mice showed a significant increase in the latency to fall compared to wild type at ages 12 and 18 months (**$p<0.01$, two-way ANOVA, Tukey's post-hoc for both *Parkin*$^{-/-}$*DJ-1*$^{-/-}$ and *Parkin*$^{-/-}$*DJ-1*$^{-/-}$*Gpx1*$^{-/-}$), with genotype and trial as factors.

The surprising improvement in rotarod performance of *Parkin*$^{-/-}$*DJ-1*$^{-/-}$ mice and *Parkin*$^{-/-}$*DJ-1*$^{-/-}$*Gpx1*$^{-/-}$ prompted me to re-examine the rotarod performance of *Parkin*$^{-/-}$ mice and *DJ-1*$^{-/-}$ mice, especially because they had been backcrossed from a hybrid to a pure C57BL/6 genetic background since our previous studies (Goldberg et al., 2003, Goldberg et al., 2005). I compared the rotarod performance of wild-type, *Parkin*$^{-/-}$, *DJ-1*$^{-/-}$ and *Parkin*$^{-/-}$ *DJ-1*$^{-/-}$ mice in young (6 month) and aged (13 and 16 month) cohorts. At ages 6, 13 and 18 months, *Parkin*$^{-/-}$*DJ-1*$^{-/-}$ mice perform significantly better than wild type mice ($p \leq 0.001$, two-way repeated measures ANOVA). Therefore, the improvement observed in *Parkin*$^{-/-}$*DJ-1*$^{-/-}$ and *Parkin*$^{-/-}$*DJ-1*$^{-/-}$*Gpx1*$^{-/-}$ mice may be due to a synergistic effect of *DJ-1* and *Parkin* deficiencies and further rotarod testing considered *Parkin*$^{-/-}$*DJ-1*$^{-/-}$ and *Parkin*$^{-/-}$*DJ-1*$^{-/-}$*Gpx1*$^{-/-}$ mice to be equivalent (Figure 4-15). However, the exact reasons why the double mutant mice remain on the rotarod longer than wild type mice remain to be identified.

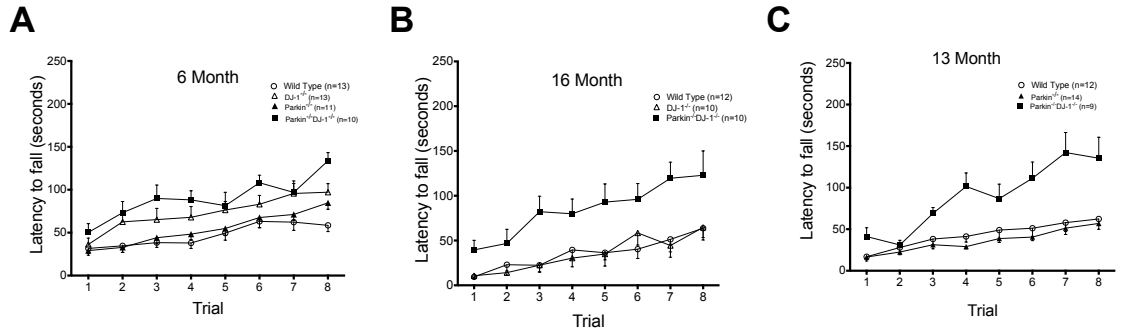

Figure 4-16. *Parkin*$^{-/-}$*DJ-1*$^{-/-}$ mice have improved rotarod performance. The latency to fall off an accelerating rotating rod was measured for separate cohorts of mice at age 6 (A), 16 (B) and 13 months (C), n=9-13 mice per genotype. Symbols represent the mean ± SEM time (seconds) before falling off the rod for each of 8 trials. While all genotypes learned the task over multiple trials, *Parkin*$^{-/-}$*DJ-1*$^{-/-}$ mice showed a significant increase in the latency to fall compared to wild type at ages 16 and 13 months (**$p<0.01$, two-way ANOVA, Tukey's post-hoc, with genotype and trial as factors).

No statistically significant differences were observed in anxiety levels as measured by open field, dark light box elevated plus maze, indicating that altered anxiety cannot explain the rotarod behavior differences (Figures 4-17, 4-18, 4-19). These data suggest that *Parkin*$^{-/-}$ *DJ-1*$^{-/-}$ and *Parkin*$^{-/-}$*DJ-1*$^{-/-}$*Gpx1*$^{-/-}$ mice show age-dependent improvement in rotarod performance compared to *Gpx1*$^{-/-}$ and wild type mice.

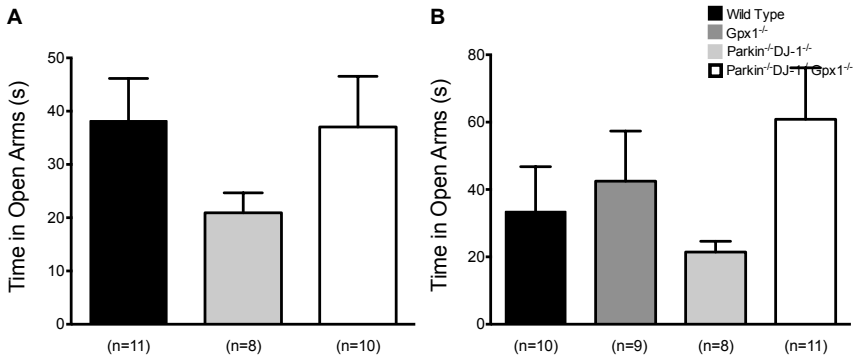

Figure 4-17. Anxiety, as measured by time spent in the open arms of an elevated plus maze, was not different between PD mutant and wild type mice at ages 12 (A) and 18 (B) months. Bars represent mean ± SEM seconds in open arms. ($p\geq0.2$, one-way

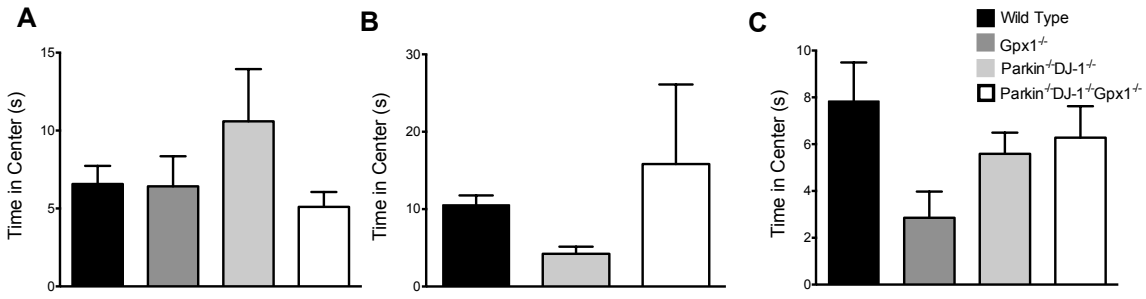

Figure 4-18. Anxiety, as measured by time spent in the center of an open field test, was not different between genotypes at ages 6 (A), 12 (B) and 18 (C) months. Bars represent mean ± SEM seconds spent in the center. ($p \geq 0.0.08$, one-way ANOVA).

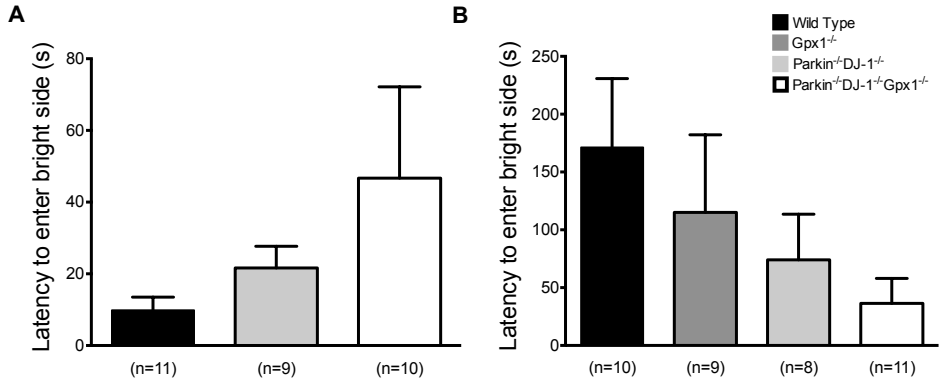

Figure 4-19. Anxiety, as measured by time to enter the bright side of a dark/light box, was not different between genotypes at ages 12 (A) and 18 (B) months. Bars represent mean ± SEM seconds to enter bright side. ($p \geq 0.19$, one-way ANOVA).

To investigate whether the increased rotarod latencies in *Parkin*$^{-/-}$*DJ-1*$^{-/-}$ were due to improved motor skills or non-motor aspects of this test, I measured the latencies of fully trained 8-month-old wild-type and mutant mice to fall off the rotarod at fixed speeds of 5, 10,

15 and 20 RPM for 5 min (Figure 4-20). Wild-type mice fell off the rotarod significantly faster than *Parkin*$^{-/-}$, *DJ-1*$^{-/-}$ and *Parkin*$^{-/-}$*DJ-1*$^{-/-}$ mice at all speeds tested, including low speeds, such as 5 and 10 RPM, that did not challenge the motor abilities of the mice.

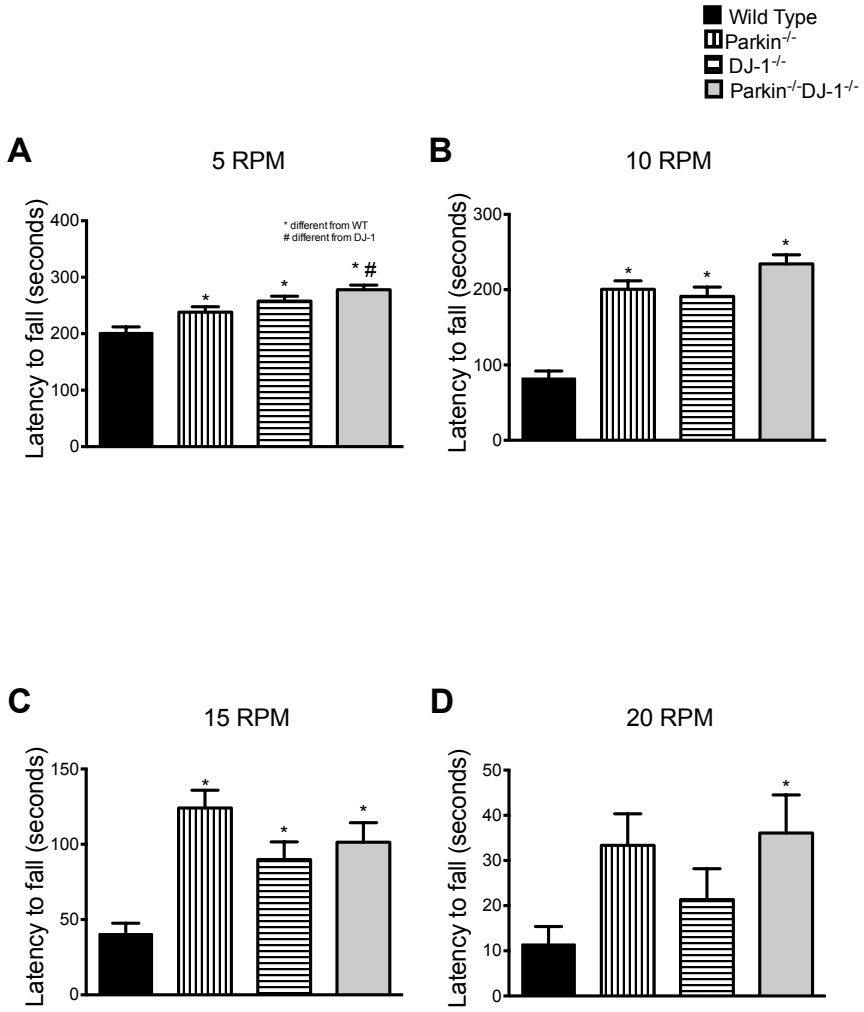

Figure 4-20. Improved motor and non-motor skills in *Parkin*$^{-/-}$, *DJ-1*$^{-/-}$ and *Parkin*$^{-/-}$*DJ-1*$^{-/-}$ mice compared to wild type. A cohort of mice at age 8 months was fully trained to perform the rotarod test and then tested at fixed rotarod speeds of 5 (A), 10 (B), 15 (C) and 20 rotations per minute (RPM) (D). Bars represent the mean ± SEM latency to fall off the rotating rod. The single and double knock-out mice showed increased latency to fall compared to wild type (*$p<0.0001$, one-way ANOVA, Tukey's post-hoc). *Parkin*$^{-/-}$*DJ-1*$^{-/-}$ mice also showed increased latency to fall compared to *DJ-1-/-* at the lowest speed (A).

I also analyzed video recordings of mice during the fixed-speed rotarod test. It was apparent that all mice could easily perform the task at slow speeds and that the mice that fell off did so upon turning around or exploring the left or right sides of the rod rather than facing forward. An investigator blind to genotype analyzed the videos with a stopwatch and measured the time each mouse was not facing forward, which was considered "distracted" from the task, during the first 30 seconds of the fixed-speed rotarod test. At 10, 15 and 20 RPM, *Parkin*$^{-/-}$ *DJ-1*$^{-/-}$ mice spent significantly less time "distracted" on the rotarod compared to wild type mice (p<0.05-0.001, one-way ANOVA) (Figure 4-21). At 5 RPM, the same trend was observed, likely accounting for the longer latencies to fall compared to wild-type mice.

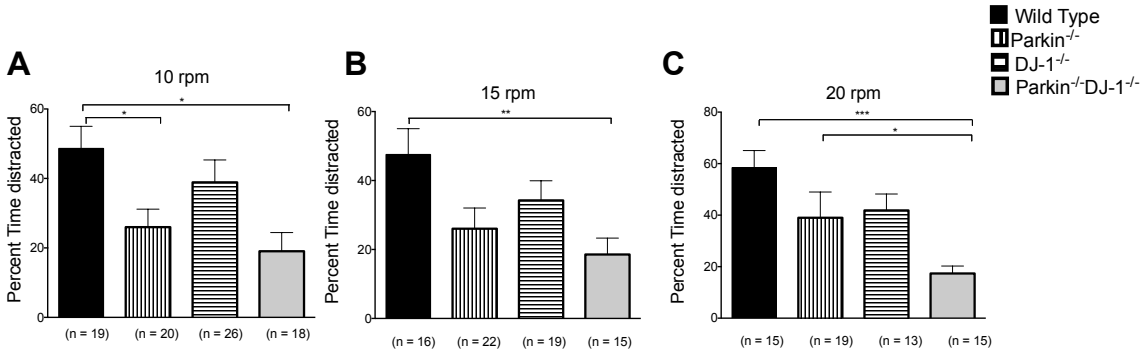

Figure 4-21. *Parkin*$^{-/-}$, *DJ-1*$^{-/-}$ and *Parkin*$^{-/-}$*DJ-1*$^{-/-}$ mice display less "distraction" behavior compared to wild type mice. A cohort of mice at age 8 months was fully trained to perform the rotarod test and was videotaped during testing at fixed speeds of 10, 15 and 20 RPM. Video of the fixed-speed rotarod behavior was analyzed to measure the percent of time each mouse was not facing straight forward on the rotarod apparatus as a surrogate measure of "distraction" (A-D). Bars show mean ± SEM percent time on the rotarod not facing forward *Parkin*$^{-/-}$*DJ-1*$^{-/-}$ mice spent more time facing forward at 5 (A), 10 (B), 15 (C) and 20 rotations per minute (RPM) compared to wild type (*p<0.05, **p<0.01, and ***p<0.001, one-way ANOVA, Tukey's post-hoc).

This decreased distraction may be a form of increased attention. Therefore, I examined whether the differences in rotarod behavior are due to changes in attention. To accomplish this I administered drugs used to treat attention deficit disorder (ADD), namely methylphenidate (MPH), yohimbine and guanfacine. MPH effectively increases dopamine signaling within the brain. Both yohimbine and guanfacine act on the adrenergic system, yohimbine as an α_{2A}-adrenergic receptor antagonist, and guanfacine as an agonist. Administration of MPH and yohimbine had no effect on the differences between the two genotypes ($p < 0.01$, two-way ANOVA) (Figure 4-22A, C). Similarly, administration of guanfacine had little effect on the rotarod performance in either wild type or *Parkin*$^{-/-}$*DJ-1*$^{-/-}$ mice ($p < 0.01$, two-way ANOVA), although by the fourth trial of the day, neither group was able to stay on the rotarod for longer than an average latency to fall of 35 seconds, most likely due to the sedative effects of this drug (Figure 4-22B). Taken together, these results indicate that administration of drugs used to treat ADD and improve attention do not affect the differences observed in rotarod performance in the double mutant mice compared to wild type, thus suggesting that rotarod differences are not due to changes in attention controlled by either the dopaminergic or adrenergic neurotransmitter systems. Instead, the improvement on the rotarod task may be due to other changes not related to dopaminergic or adrenergic neurotransmitter systems or attention.

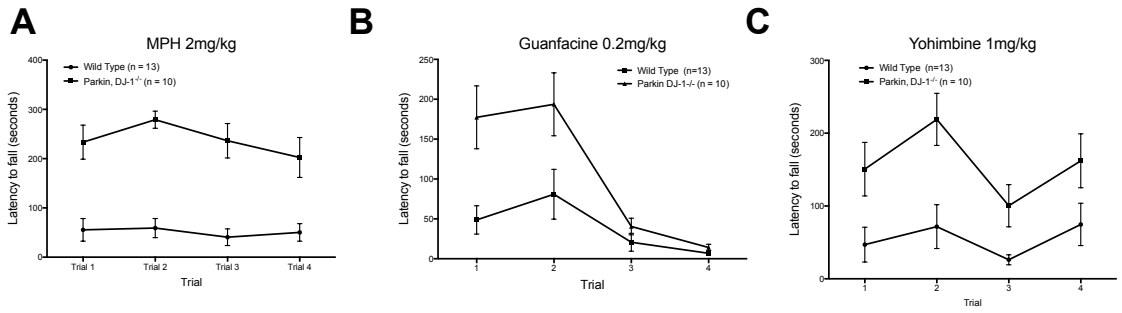

Figure 4-22. Administering attention deficit disorder medication to wild type and *Parkin*$^{-/-}$*DJ-1*$^{-/-}$ mice does not affect rotarod performance. A cohort of mice at age 8 months was fully trained to perform the rotarod test and then tested after administration of drugs used to treat ADD. Bars represent the mean ± SEM latency to fall off the rotating rod. The double knock-out mice showed increased latency to fall compared to wild type (*$p<0.0001$, two-way ANOVA, with genotype and age as factors).

The results from the rotarod experiments were also examined for changes in learning the rotarod task over time. Learning was calculated as the change in latency to fall over time. Therefore, early rotarod perfomance was compared to later rotarod performance by subtracting the average latency to fall in the first two trials from average latency to fall in the last two trials. This comparison shows that the double mutant mice have a greater difference in latency to fall between the first and last two trials, indicating that double mutant mice exhibit greater learning over the course of eight trials (Figure 4-23) ($p \leq 0.03$, Student's t-test).

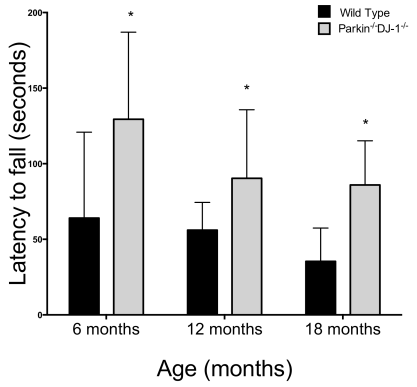

Figure 4-23. Improved motor learning in *Parkin*$^{-/-}$*DJ-1*$^{-/-}$ mice. Latency to fall (in seconds) from the rotarod in the first trial was subtracted from latency to fall in the last trial at 6, 12 and 18 months. Bars represent the mean ± SEM latency to fall off the rotating rod. The double knock-out mice showed increased latency to fall compared to wild type (*$p<0.0001$, two-way ANOVA, with genotype and age as factors).

Mutant mice had comparable anxiety to wild type mice

Another possible explanation for the improved rotarod performance may be an increase in anxiety in the mutant mice, which may cause affected animals to work harder to remain on the rotarod. Therefore, mice were tested for an anxiety phenotype in a variety of paradigms, including open field, elevated plus maze and the dark/light test at two different ages: 12 and 18 months old. Mice aged 6 months old were also tested in the open field. However, no genotypes exhibited a consistent anxiety phenotype compared to wild type mice. Wild type mice spent significantly more time in the center at 12 months in the open field test, by one-way ANOVA ($p=0.0012$), but no significant differences were observed between any genotypes at either 6 or 18 months of age ($p > 0.2$) (Figure 4-18). In the elevated plus maze, no differences were found between any of the genotypes at any age, by one-way ANOVA (p

> 0.1). Although the *Parkin*$^{-/-}$*DJ-1*$^{-/-}$ mice do show a trend toward decreased time in the open arm, this difference was not found to be significant (Figure 4-17). Finally, no significant differences were found between any of the genotypes in the dark/light test by one-way ANOVA ($p \geq 0.1$), although the *Parkin*$^{-/-}$*DJ-1*$^{-/-}$*Gpx1*$^{-/-}$ mice exhibit a trend toward decreased time in the bright side and decreased time to enter the bright side (Figure 4-19). Overall, all mice were considered to have similar anxiety levels to those in wild type mice due to a lack of a consistent anxiety phenotype.

Increased acoustic startle response in PD mutant mice compared to wild type

In order to test for alterations in responses to stimuli, such as a loud noise, the level of startle in mice in response to tones set to varying loudness was measured. All mice exhibit increased startle in response to increasingly loud stimuli. However, compared to wild type mice, the *Parkin*$^{-/-}$*DJ-1*$^{-/-}$*Gpx1*$^{-/-}$ mice exhibit decreased startle response at 12 months of age ($p=0.01$, two way ANOVA) while the *Parkin*$^{-/-}$*DJ-1*$^{-/-}$ mice show decreased startle response at both 6 and 12 months of age ($p=0.01$, two way ANOVA). No differences were observed in the 18 month old mice, although this observation may be due to an overall decrease in response, even in the wild type mice (Figure 4-24).

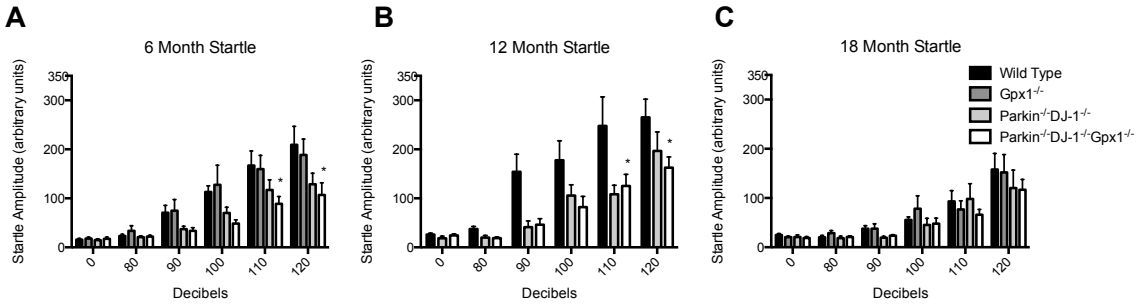

Figure 4-24. *Parkin*$^{-/-}$*DJ-1*$^{-/-}$ and *Parkin*$^{-/-}$*DJ-1*$^{-/-}$*Gpx1*$^{-/-}$ show decreased startle response compared to wild type. Overall, mice exhibit increased startle in response to increasing intensity of the auditory stimulus ($p<0.0001$, two way ANOVA with simulus intensity and genotype as factors). *Parkin*$^{-/-}$*DJ-1*$^{-/-}$ *Gpx1*$^{-/-}$ show a decreased startle response at 110 and 120 dB at 6 months of age (A) and at 12 months of age (B). *Parkin*$^{-/-}$*DJ-1*$^{-/-}$ also exhibit increased startle response at 12 months at decibels of 110 and 120 (B). No differences were observed between genotypes at 18 months (C). Bars represent the mean ± SEM of the force of the startle response. (*$p=0.039$, *$p=0.013$, $p=0.59$, respectively, by two-way ANOVA with stimulus intensity and genotype as factors)

Normal olfactory function in PD mutant mice

In order to rule out potential sensory deficits as a cause of altered rotarod behavior, I tested mice for olfactory function because one common preclinical symptom of PD is anosmia (Doty et al., 1988, Doty et al., 1992, Pellicano et al., 2007). I hypothesized that decreased olfactory function may cause *Parkin*$^{-/-}$*DJ-1*$^{-/-}$ mice to explore less and consequently show longer latencies to fall off of the rotarod. However, I found no evidence of impaired olfactory function in mutant mice (Figure 4-25). These data suggest that the improved rotarod performance of *Parkin*$^{-/-}$*DJ-1*$^{-/-}$ mice is not attributable to a lack of olfactory distractions.

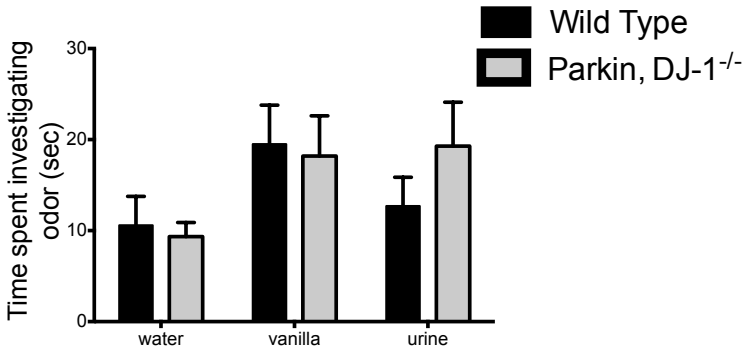

Figure 4-25. Parkinson's disease mutant mice have intact olfactory function. Mice were tested for the amount of time spent investigating novel odors of vanilla and urine from unfamiliar mice. Although both groups of mice showed increased time examining the vanilla and urine, compared to water (p=0.0099) *Parkin*$^{-/-}$*DJ-1*$^{-/-}$ and wild type mice showed comparable olfactory function (p=0.757 two-way ANOVA, with genotype and scent as factors). Bars represent mean ± SEM time spent investigating the odor during

The mice in these studies generally appear normal and healthy, with no PD-related deficits in behavior. However, double and triple mutant mice exhibit unexpected improvement on the rotarod test while double mutant mice have increased striatal serotonin and triple mutant mice have increased striatal dopamine. The mice do not have changes in locomotor tests or in the number of neurotransmitter-producing neurons, suggesting that behavior changes are independent of motor ability and that increases in neurotransmitters occurs on a sub-cellular level.

CHAPTER FIVE

DISCUSSION

Although mice deficient for multiple genes related to Parkinson's disease do not exhibit classical symptoms of PD, the unexpected behavioral and neurochemical changes indicate these PD-related genes have important functions beyond the dopaminergic nigrostriatal circuit.

Discussion

Absence of parkinsonian behavioral phenotypes

In characterizing parkinsonian phenotypes in mice with mutations linked to PD, I analyzed H_2O_2 levels, a major source of reactive oxygen species, and antioxidant protein levels, specifically glutathione peroxidase 1 and superoxide dismutases 1 and 2. I found no changes in these antioxidant systems in brains of mice deficient for genes linked to Parkinson's disease. Furthermore, there were no changes in oxidative damage as measured by oxyblot. This is in contrast to the expected increase in reactive oxygen species and oxidative damage. The lack of responsiveness in mice to loss of antioxidant genes indicates that mice may either have different mechanisms for handling the loss of antioxidant genes or that PD linked genes do not have the same role or effect on the brains of mice that they have in the brains of

humans, although I cannot rule out that other antioxidant mechanisms, not examined here, compensate for the loss of these genes in mice.

Absence of classical PD symptoms in PD mutant mice

These mice were also tested for classical symptoms of PD, including nigral cell loss. There are no reported postmortem examinations of cases of PD linked to *DJ-1* mutations, but autopsies of *Parkin*-linked PD consistently show profound loss of nigral dopaminergic neurons. Evidently, deletion of both *Parkin* and *DJ-1* in mice is not sufficient to induce nigral cell loss because I did not observe significant differences in the number of nigral dopaminergic neurons measured by unbiased stereology. *Gpx1* deficiency has previously been shown to increase vulnerability to MPTP-induced nigral cell loss (Klivenyi et al., 2000, Zhang et al., 2000); therefore I expected $Parkin^{-/-}DJ\text{-}1^{-/-}Gpx1^{-/-}$ mice to have an age-dependent decrease in nigral dopaminergic neurons, resulting in depletion of striatal dopamine. Instead, I observed a significant increase in the levels of striatal dopamine in $Parkin^{-/-}DJ\text{-}1^{-/-}Gpx1^{-/-}$ mice, but not in $Gpx1^{-/-}$ or $Parkin^{-/-}DJ\text{-}1^{-/-}$ mice.

Species differences may explain lack of parkinsonian phenotypes in mice

It has been well documented that certain cellular mechanisms in mice are different from equivalent mechanisms in humans. For example, glutathione peroxidase levels decrease with age in the brains of humans (Venkateshappa et al., 2012), but not in the brains of mice (Benzi et al., 1989). There are other examples of differences between mice and humans, including a decrease in alpha synuclein levels with age in mice, in contrast to reports that alpha synuclein

is increased in the brains of humans and primates with age (Mak et al., 2009). Therefore, it is possible that additional differences between mice and humans in response to PD linked mutations could explain the lack of a parkinsonian phenotype in the mice examined here. These differences could be informative for developing PD treatments because mice do not exhibit parkinsonian symptoms over time. Unfortunately, at this point little is known about what exactly causes mice to be resistant to PD-related neurodegeneration.

These data are in agreement with results from many other studies indicating that genetic manipulation in mice does not result in PD symptoms. Multiple studies have determined that loss of either *Parkin* or *DJ-1* in mice does not result in a classical PD phenotype (Goldberg et al., 2003, Fleming et al., 2005, Perez and Palmiter, 2005, Hattori and Sato, 2007, Chandran et al., 2008, Kitada et al., 2009, Dawson et al., 2010), although dysfunctional mitochondria have been reported and may be an early indication of parkinsonian symptoms. This leads to the question of why additional symptoms are not seen in mice, especially at the oldest ages. Possibly the lack of additional symptoms is due to the limited lifespan in mice because mice typically do not live longer than 24 months. I find this explanation to be unlikely, however, because recent genetic models of rats exhibit parkinsonian symptoms including loss of nigral neurons within a two-year life span (data not published). Additionally, at least one study (Kitada et al., 2009) in which triple mutant mice for *Parkin*, *DJ-1* and *PINK1* are found to have increased striatal dopamine in the absence of another phenotype has determined that combined mutations for PD do not result in parkinsonian phenotypes. *PINK1* acts in the same pathway as *Parkin* and is believed to recruit *Parkin* to impaired mitochondria (Narendra et

al., 2008). Possibly, the loss of PINK1 in addition to *Parkin* and *DJ-1* increases mitochondrial free radical production in aged mice, thus acting as a stressor in a similar manner to loss of *Gpx1* and resulting in a similar phenotype. Together with my data, this indicates that *Parkin* and *DJ-1* are involved in regulating neurotransmitters.

Increased neurotransmitter levels in PD mutant mice

The increased dopamine in triple mutant mice and the increased serotonin in double mutant mice is very surprising. Each of these findings has been replicated at different ages and has been found to be highly reproducible in multiple cohorts of mice. One possible explanation for these findings may be a compensatory response in to loss of dopaminergic terminals in the striatum prior to nigral neuron loss similar to the retrograde neurodegeneration thought to occur in PD patients (Bernheimer et al., 1973) and observed in mouse and rat models following intrastriatal 6-hydroxydopamine injections, rotenone treatment or loss of mitofusin2 (Lee et al., 1996, Cannon et al., 2009, Pham et al., 2012). Alternatively, all three genes may interact to affect dopamine production, trafficking, release, degradation, or pre-synaptic or post-synaptic signaling, resulting in a net increase in steady-state dopamine levels. In support of this, both *Parkin* and *DJ-1* have been found to be located in the membranes of synaptic vesicles (Kubo et al., 2001, Usami et al., 2011) and mice with reduced vesicular monoamine storage have been shown to exhibit nonmotor symptoms of PD, which may be related to early deficiencies in PD (Taylor et al., 2009, Vernon, 2009). Furthermore, altered dopamine release, reuptake and synaptic plasticity within the striatum have been observed in *Parkin*$^{-/-}$ mice and *DJ-1*$^{-/-}$ mice (Jiang et al., 2004, Goldberg et al.,

2005, Kitada et al., 2009, Jiang et al., 2012). Elevated striatal dopamine levels may compensate for early stage defects in dopaminergic signaling and it is possible that without this compensation there would be detectable locomotor behavioral deficits in *Parkin*$^{-/-}$*DJ-1*$^{-/-}$*Gpx1*$^{-/-}$ mice. Alternatively, the increased striatal dopamine may cause behavioral phenotypes that are not detected by our tests of locomotor function.

In addition to a surprising increase in dopamine levels, I also found a surprising increase in serotonin levels in both the striatum and the hippocampus of *Parkin*$^{-/-}$*DJ-1*$^{-/-}$ mice, indicating that loss of two genes was sufficient to observe increases in serotonin whereas the additional loss of *Gpx1* was required to observe changes in dopamine levels. The increase in serotonin was also observed in multiple brain regions, suggesting that the effects of *Parkin* and *DJ-1* deficiency are widespread. This is in contrast to the decrease in serotonin and serotonin transporter (SERT) seen at the end stage in PD patients (Scatton et al., 1983, Politis et al., 2010a, Politis et al., 2010b, Roselli et al., 2010). Interestingly, however, transgenic mice expressing mutated human alpha-synuclein linked to PD have increased SERT by western blot, suggesting increased serotonin levels (Graham and Sidhu, 2010, Yamakado et al., 2012), while MPTP-treated primates have serotonergic hyperinnervation following dopamine loss (Zeng et al., 2010); these finding are in agreement with the increase in serotonin that I describe here. Therefore, increased serotonin is likely due to changes in the brain influenced by genetic alterations linked to PD. In fact, decreased dopaminergic innervation may result in an initial compensatory increase in serotonergic innervation (Kish et al., 2008). Because both dopamine and serotonin are monoamine neurotransmitters and share metabolic processes,

and because transporters may be capable of taking up either molecule, it has been previously suggested that increased serotonin compensates for loss of dopamine (Norrholm et al., 2007, Boulet et al., 2008, Bedard et al., 2011, Morelli et al., 2011). Overall, these changes in both neurotransmitter levels indicate compensatory responses in the brain and a misregulation of the neurotransmitter system, possibly prior to disease onset caused by deficits of *Parkin* and *DJ-1* genes. Additionally, due to similarities between dopamine and serotonin, the increase in serotonin may be a compensatory response to dopaminergic degeneration not visible by measuring the numbers of dopamine-producing neurons.

Increases in neurotransmitter levels can result from increased neurotransmitter-releasing fibers. I hypothesized that the significant increase in hippocampal serotonin levels in *Parkin*$^{-/-}$*DJ-1*$^{-/-}$ mice is due to an increase in hippocampal serotonergic fibers. Although I observed a trend towards increased hippocampal SERT-positive fibers in *DJ-1*$^{-/-}$ and *Parkin*$^{-/-}$*DJ-1*$^{-/-}$ mice, they are not significantly different from wild-type and *Parkin*$^{-/-}$ mice. It is possible that older mice would show a greater difference in SERT-positive fiber staining. However, I believe this to be unlikely because significant differences in neurotransmitter levels are observed as early as 6 months by t-test.

Alternatively, an increase in tryptophan hydroxylase (TpH), which is the enzyme in the rate-limiting step of serotonin production, may cause an increase in serotonin levels. Therefore, I measured hippocampal TpH expression levels by quantitative PCR (QPCR) after reverse transcription of total RNA. However, no differences were found between TpH levels in

Parkin$^{-/-}$*DJ-1*$^{-/-}$ mice compared to single mutant *Parkin*$^{-/-}$ and *DJ-1*$^{-/-}$ mice, or compared to wild type mice. This indicates that increases in serotonin are not due to increases in TpH expression. However, increases in activity levels of TpH, which were not measured, could be responsible for increased neurotransmitter levels.

Mice lack behavioral parkinsonian phenotypes

Motor skills

Rotarod performance is routinely used to assess motor function in rodent models of neurodegenerative disease. Our data indicate that mice deficient for *Parkin* and *DJ-1* do not have motor impairment but instead show improved performance on the rotarod task compared to controls. Since the improvement in rotarod performance occurs in double mutant mice, but increased dopamine only occurs in the triple mutant mice, it is unlikely that changes in dopamine levels can explain the improved rotarod behavior. Moreover, it has been previously noted that a loss of dopamine does not necessarily result in decreased rotarod performance (Zurkovsky et al., 2013), suggesting that rotarod behavior is affected by other factors. Therefore, I examined other possible explanations for the observed differences in rotarod behavior. I determined that the increase in rotarod performance is not explained by an overall increase in activity because spontaneous locomotor behavior was unchanged in *Parkin*$^{-/-}$*DJ-1*$^{-/-}$ mice compared to controls. Previous studies have shown that other factors can contribute to rotarod performance, such as body weight or sensory abilities (McFadyen et al., 2003). However, *Parkin*$^{-/-}$*DJ-1*$^{-/-}$ mice do not exhibit changes in olfactory function, a common non-motor symptom of PD. Differences in weight are unlikely to account for the

differences in rotarod behavior because wild type mice are, on average, larger than all other genotypes but perform no differently from *Parkin*$^{-/-}$, *DJ-1*$^{-/-}$ and *Gpx1*$^{-/-}$ mice despite the difference in weight. Improved rotarod performance could also be indicative of cognitive changes in mice lacking both the *Parkin* and *DJ-1* genes. For instance, rotarod performance can be affected by learning ability, and the double and triple mutant mice in this study have a greater difference between performance on the last trial compared to the performance on the first trial (total time last trial – total time first trial) than do either wild type mice or *Gpx1* single mutant mice. This indicates either that the double and triple mutant mice learn faster than the wild type and single mutants, resulting in a greater improvement in performance, or that double and triple mutant mice continue to learn the task over the eight trials whereas wild type and single mutants stop learning after the first few trials. Alternatively, the wild type and single mutants may learn to fall more quickly and end the trial sooner.

Related to this difference in learning, I noticed anecdotally that double and triple mutant mice seemed to be more attentive to their tasks. This observation was also evident in the digigait trials because data collection for the double and triple mutant mice was significantly easier due to their lack of exploratory behavior while on the treadmill. It should be noted that this behavior was only observed during times of forced locomotion (digigait and rotarod) but not during times of voluntary locomotion (locomotor and anxiety tests). Therefore, I tested the mice on a fixed speed rotarod test to rule out the possibility that the task was too difficult for the wild type and mice. The results of these experiments showed that, even at speeds as low as 5 RPM, wild type mice fell off the rotarod sooner than *Parkin*$^{-/-}$*DJ-1*$^{-/-}$ mice,

suggesting that motor ability does not fully account for the deficiencies in rotarod performance. This prompted me to measure distraction behavior during rotarod testing, which were found to be increased in the *Parkin*$^{-/-}$*DJ-1*$^{-/-}$ mice compared to wild type.

To further test the possibility that attentiveness can affect the outcome of the rotarod test, I administered medications commonly used for the treatment of attention deficit disorder (ADD), guanfacine, yohimbine and methylphenidate (MPH), to both wild type and *Parkin*$^{-/-}$ *DJ-1*$^{-/-}$ mice prior to testing them on a fixed speed rotarod. Yohimbine has depressant effects, and was found to effectively put the mice to sleep, even at very low doses, and therefore I did not use it for further experimentation. Guanfacine and methylphenidate had limited effects on either the wild type or *Parkin*$^{-/-}$*DJ-1*$^{-/-}$ mice, even at high doses of MPH, suggesting that the mechanisms by which these drugs increase attentiveness, namely by potentiating dopaminergic signaling in the case of MPH, acting as a norepinephrine agonist in the case of yohimbine, or activating alpha-2 adrenergic (α2A) signaling by guanfacine, do not cause the improved rotarod behavior in these mice.

Startle Response

In addition to alterations in rotarod performance, *Parkin*$^{-/-}$*DJ-1*$^{-/-}$ and *Parkin*$^{-/-}$*DJ-1*$^{-/-}$*Gpx1*$^{-/-}$ mice have decreased startle response. Although similar mutants, *SOD2*$^{+/-}$ and *Parkin*$^{-/-}$*DJ-1*$^{-/-}$ *SOD2*$^{+/-}$ mice, also have decreased startle response due to hearing loss (McFadden et al., 1999, Hennis et al., Submitted), it is unlikely that this could explain the decreased response in the double mutant mice discussed here because these mice have not been reported to

exhibit hearing loss. Interestingly, *Gpx1* knockout mice are reported to have hearing loss (Ohlemiller et al., 2000), however, the *Gpx1* single knockout mice do not exhibit decreased startle response in contrast to the *Parkin*$^{-/-}$*DJ-1*$^{-/-}$ double mutant mice that do have changes in startle response. This suggests that the decreases in startle response are due to the combination of *Parkin* and *DJ-1* deficiency and that loss of *Gpx1* does not have a significant effect in our study. Motor issues are also unlikely to explain this phenotype because locomotor tests aside from the rotarod test, failed to discern a motor phenotype in these mice. These results might be explained by the decreased average weight in the mutant mice, however wild type mice and *Gpx1*$^{-/-}$ mice have similar startle responses despite differences in weight, suggesting, similar to the rotarod outcomes, that this cannot fully explain the observed phenotype.

Absence of an anxiety or olfactory phenotype

An alternative explanation for the improved rotarod performance might be increased anxiety in the mutant mice, resulting in an increased latency to fall from the rotarod because mice with increased anxiety may be more fearful of falling and will therefore work harder to remain on the rod. However, no consistent change in an anxiety phenotype was observed in any of the lines of mutant mice, suggesting that deficiencies of *Parkin* and *DJ-1* do not affect anxiety levels. Classical measures of symptoms of PD are based on the decline in motor skills and do not include measures of anxiety. Despite this, anxiety has been reported as a prodromal symptom of PD (Bonnet and Czernecki, 2013), and like other symptoms, such as depression, sleep disturbances, and other non-motor symptoms, are highly variable and do

not appear in all cases of PD. However, because these mice do not exhibit altered anxiety symptoms, they should not be considered models of parkinsonian anxiety. Moreover, increased anxiety does not explain the improved performance on the rotarod.

Anosmia has been reported as an early symptom of PD (Doty et al., 1988, Doty et al., 1992) and *PINK1* mutant mice have been reported to have olfactory deficits (Glasl et al., 2012). Therefore, I hypothesized that the decreased distraction on the rotarod test may be due to sensory deficits that cause the mice to explore their environment to a lesser extent than the wild type mice. However, both the double mutant mice and wild type mice spent more time examining novel odors and the double mutants performed no differently than the wild type mice. Therefore, the double mutants do not appear to have anosmia and it is unlikely that decreased distraction is due to a sensory deficit.

Increased neurotransmitter levels do not explain behavioral changes

Although I have seen changes in both dopamine levels and motor behavior in the mice examined here, these two observations are not necessarily related to one another because the increase in striatal dopamine is observed in *Parkin*$^{-/-}$*DJ-1*$^{-/-}$*Gpx1*$^{-/-}$ mice while the improved rotarod behavior is observed in *Parkin*$^{-/-}$*DJ-1*$^{-/-}$ mice, indicating that a loss of all three genes is required in order to observe increased dopamine levels, but deficiencies in only two genes: *Parkin* and *DJ-1* is sufficient to see increased latency to fall from the rotarod. However, the increased striatal and hippocampal serotonin is observed in *Parkin*$^{-/-}$*DJ-1*$^{-/-}$ mice, the same genotype in which I see improved rotarod performance. Previously published studies have shown that mice with serotonergic system disruption have decreased performance on the

rotarod test, but these changes typically occur in conjunction with changes in basal locomotor activity (Holmes et al., 2002a, Holmes et al., 2002b, Morelli et al., 2011). However, changes in serotonin levels have been shown to alter cognitive abilities, such as attention and learning (Buhot, 1997) and thus may account for the changes observed in the rotarod test because mutant mice are observed to have both increased learning as well as increased attention. Therefore, it is possible that the altered rotarod behavior is related to the increased hippocampal serotonin.

Future Directions

Although the mice discussed here do not have the classical motor symptoms of PD, these mice could be considered either an early, pre-symptomatic model of PD, or simply provide insight into the functions of *Parkin* and *DJ-1*. Specifically, they are informative for understanding how the functions of *Parkin* and *DJ-1* affect the neurotransmitter systems beyond both the dopamine system and the striatum. In order to take full advantage of this pre-symptomatic model of PD, further studies are required to examine both the altered behavior of the mice as well and the mechanisms by which the neurotransmitter systems are affected and whether or not these two phenotypes are linked.

Although several functions of *Parkin* and *DJ-1*, including involvement in synaptic membranes and dopamine release (Oyama et al., 2010, Usami et al., 2011) have been published, the exact mechanisms by which loss of a functional copy of either of these genes results in PD remains to be elucidated. The mice in this study offer the unique opportunity to

study the ways in which *Parkin* and *DJ-1* can have an effect on neurotransmitter systems involved in PD because these mice have a consistent increase in total levels of dopamine and serotonin, both of which are known to be decreased in PD. In the future, I would want to know whether any other genes related to neurotransmitter production, release or reuptake are up-regulated or down-regulated in mice deficient for *Parkin*, *DJ-1* and *Gpx1*. I have begun to look at some of these genes, such as TpH and TH, by quantitative PCR, and have seen a trend toward increased expression levels, but this does not fully explain the increase in neurotransmitter levels. Therefore, I would be interested in examining whether there is an increase in transporter levels as well, especially given previous reports that *Parkin* and *DJ-1* are involved in synapses and dopamine release. A broader microarray analysis might also be useful for determining other pathways that are affected by deficiencies of these genes. To date, I am unaware of this kind of study in *Parkin*$^{-/-}$ mice, although gene expression profiling has been performed on cells lacking DJ-1 or containing the disease-linked L166P mutation and have found altered expression of the tau gene, which is involved in Alzheimer's disease tangle pathology (Nishinaga et al., 2005). Because *Parkin* and *DJ-1* functions are unclear, loss of these genes in combination with *Gpx1* might result in altered expression of yet other genes that may provide insights into the development PD.

In order to determine whether there is a causal link between the effects of *Parkin* and *DJ-1* deficiency and the observed behaviors, it would be informative to pharmacologically alter the serotonergic system prior to testing mice on the rotarod again. Candidate drugs include para-chloropheynlalanine (PCPA), an inhibitor of tryptophan hydroxylase that would reduce the

amount of available serotonin, as well as serotonin receptor agonists or antagonists. Therefore, if increased serotonin causes the improved rotarod performance, the *Parkin*$^{-/-}$*DJ-1*$^{-/-}$ mice would be expected to have performance levels similar to wild type following drug administration.

Additionally, I would like to know whether the increased rotarod latency in *Parkin*$^{-/-}$ *DJ-1*$^{-/-}$ mice is due to attention or motivation because an increase in either of these could result in the decreased distraction that I observed in this study. Attention can be measured using a 5-choice serial reaction time test, in which the time and accuracy of the animal's nosepoke in response to a visual stimulus is measured. However, this test is very difficult to conduct in mice. Alternatively, measuring the motivational and perseverative behavior in mice by quantifying how much the mice are willing to nosepoke for a reward is an easier test to perform and would be informative for determining the ways that loss of *Parkin* and *DJ-1* can affect the mouse's mental state, especially because serotonin is known to be involved in cognitive functions such as motivation (Roiser et al., 2006).

Conclusions

I have rigorously tested the hypothesis that loss of *Gpx1* is instrumental in the development of PD symptoms in mice bearing PD-linked mutations in *Parkin* and *DJ-1*. While adaptive changes in brain antioxidants may indeed be neuroprotective, our results demonstrate that preventing these changes by genetic disruption of *Gpx1* is not sufficient to induce nigral neuron loss in mice deficient for *Parkin* and *DJ-1*. In fact, our results indicate that *Parkin*$^{-/-}$*DJ-1*$^{-/-}$ and *Parkin*$^{-/-}$*DJ-1*$^{-/-}$*Gpx1*$^{-/-}$ mice exhibit neurochemical and behavioral

phenotypes that are contrary to the expected parkinsonian phenotype. My study demonstrates that *Parkin* and *DJ-1* mutations affect striatal serotonin levels and rotarod behavior in mice. Additionally, deficiency for all three genes causes a significant increase in striatal dopamine. It is possible that the increased striatal dopamine, striatal serotonin and hippocampal serotonin might be early-stage manifestations of nigrostriatal dysfunction induced by these mutations in mice. These results have important implications for studies of PD pathogenesis and for efforts to develop neuroprotective therapies.

APPENDIX A
Behavioral and neurotransmitter abnormalities in mice deficient for Parkin, DJ-1 and Superoxide Dismutase

Parkinson's disease (PD) is the second most common neurodegenerative disorder after Alzheimer's disease and afflicts millions of people worldwide. The primary clinical symptoms are bradykinesia, resting tremor, rigidity, and postural instability. These symptoms are caused by the loss of dopaminergic innervation of the striatum and increase in severity over time due to selective, progressive nigral dopaminergic neuron loss. Most cases of PD are sporadic and the underlying cause of neuronal death remains unknown. The greatest risk factor for PD is age. About 5 to 10% of all cases are caused by inherited mutations (Dawson et al., 2010, Horowitz and Greenamyre, 2010, Lopez and Sidransky, 2010, Corti et al., 2011, Hattori, 2012, Varcin et al., 2012). Loss-of-function mutations in the *Parkin* and *DJ-1* genes were the first mutations to be causally linked to recessive parkinsonism (Kitada et al., 1998, Bonifati et al., 2003). Both genes are widely expressed throughout the brain and other tissues (Shimura et al., 1999, Stichel et al., 2000, Kuhn et al., 2004, Shang et al., 2004, Xie et al., 2009). The mechanism by which loss of Parkin or DJ-1 function causes parkinsonism remains unclear.

Mice with targeted disruption of *Parkin* or *DJ-1* genes do not show robust neuropathology or age-dependent symptoms related to PD, suggesting the existence of compensatory mechanisms that may protect mice from the neurodegeneration and consequent motor symptoms that occur in humans with Parkin or DJ-1 mutations (Goldberg et al., 2003, Itier et al., 2003, Palacino et al., 2004, Von Coelln et al., 2004, Fleming et al., 2005, Goldberg et al.,

2005, Kim et al., 2005, Perez et al., 2005, Perez and Palmiter, 2005, Fleming and Chesselet, 2006, Sato et al., 2006, Andres-Mateos et al., 2007, Manning-Bog et al., 2007, Yang et al., 2007, Zhu et al., 2007, Chandran et al., 2008, Frank-Cannon et al., 2008, Kitada et al., 2009, Pham et al., 2010, Rousseaux et al., 2012). However, *Parkin* knockout and *DJ-1* knockout mice are more susceptible to PD-related neurodegeneration induced by various stresses including exposure to neurotoxins or to lipopolysaccharide (LPS) (Kim et al., 2005, Manning-Bog et al., 2007, Paterna et al., 2007, Frank-Cannon et al., 2008). Overexpression of Parkin or DJ-1 is neuroprotective both *in vitro* and *in vivo* (Lo Bianco et al., 2004, Zhou and Freed, 2005, Vercammen et al., 2006, Paterna et al., 2007, Ulusoy and Kirik, 2008, Hayashi et al., 2009, Junn et al., 2009, Bian et al., 2012). Parkin has been identified as an E3 ubiquitin ligase (Shimura et al., 2000)and is known to promote autophagy of dysfunctional mitochondria (Narendra et al., 2008), which are major cellular sources of free radicals and oxidative stress. Mutations in *Parkin* have been found to result in impaired mitochondrial respiration and increased markers of oxidative stress (Muftuoglu et al., 2004, Palacino et al., 2004, Rodriguez-Navarro et al., 2007, Vinish et al., 2011, Vincent et al., 2012, Hauser and Hastings, 2013, Vincow et al., 2013). The exact cellular function of DJ-1 remains uncertain, but it has been reported to be an atypical peroxiredoxin-like peroxidase (Andres-Mateos et al., 2007) and may be a sensor of oxidative stress (Choi et al., 2006). Cysteine 106 of DJ-1 is required for neuroprotection (Canet-Aviles et al., 2004, Kim et al., 2005, Junn et al., 2009, Cookson, 2010, Mullett and Hinkle, 2011, Lev et al., 2013) and is crucial for DJ-1 to localize to the mitochondria under stress conditions (Horowitz and Greenamyre, 2010, Kawajiri et al., 2010, Shulman et al., 2011, Thomas et al., 2011).

Together, these data suggest that oxidative damage is likely an important factor in the development of PD caused by *Parkin* and *DJ-1* mutations. Oxidative stress has been implicated as a potential cause of idiopathic PD because postmortem examinations of PD patients show increased oxidative damage in neurons (Dexter et al., 1989a, Alam et al., 1997, Floor and Wetzel, 1998, Zhang et al., 1999). Furthermore, the capacity of cells to clear reactive oxygen species and repair oxidative damage to proteins, lipids and nucleic acids diminishes with age (Liddell et al., 2010).

Two superoxide dismutase proteins, cytoplasmic Cu/Zn-superoxide dismutase (SOD1) and mitochondrial Mn-superoxide dismutase (SOD2) are the among the most abundant antioxidant proteins in the brain and are important for protecting neurons from oxidative stress. Some studies have suggested that abnormalities in SOD1 or SOD2 may contribute to the development of PD (Wang et al., 2011b, Belluzzi et al., 2012, Sun et al., 2012), although no mutations in *SOD1* or *SOD2* have been causally linked to PD. In flies, expression of human SOD1 is protective against neuronal loss due to inactivation of PINK1, another gene linked to recessive parkinsonism (Wang et al., 2011b). In mice, overexpression of SOD2 is protective against nigral dopamine neuron loss induced by the neurotoxin 1-methyl-4-phenyl-1,2,3,6tetrahydropyridine (MPTP) while partial SOD2-deficiency increases MPTP sensitivity (Klivenyi et al., 2000, Andreassen et al., 2001). Levels of SOD1 mRNA and SOD1 activity are significantly reduced in PD patients (Ihara et al., 1999, Kunikowska and Jenner, 2003, Boll et al., 2008). Overexpression of SOD1 or SOD2 *in vitro* and *in vivo* is protective against MPTP and 6-OHDA toxicity (Andrews et al., 1996, Asanuma et al., 1998, Hirata et al., 1998, Kunikowska and Jenner, 2003, Callio et al., 2005, Barkats et al., 2006, Lenzken et al., 2011).

MPTP exposure increases the protein levels of both SOD1 and SOD2 (Tripanichkul et al., 2007), suggesting that these proteins are important for mitigating oxidative stress in response to toxins. Together, these studies indicate that SOD1 and SOD2 protein or activity levels are key determinants of susceptibility to nigral cell loss in mice.

Aged *DJ-1*$^{-/-}$ mice have increased SOD2 in brain mitochondria, suggesting that up-regulation of antioxidant proteins may prevent PD-linked loss-of-function mutations from causing nigral cell loss in mice (Andres-Mateos et al., 2007). We hypothesize that eliminating potential compensatory upregulation of superoxide dismutase activity would result in PD-related neurodegeneration in *Parkin* and *DJ-1* knockout mice. To test this hypothesis and to potentially generate better PD animal models, we crossed mice deficient for Parkin and DJ-1 with mice deficient for SOD1 to generate triple mutant mice deficient for all three genes (*Parkin*$^{-/-}$*DJ-1*$^{-/-}$*SOD1*$^{-/-}$). We also generated *Parkin*$^{-/-}$*DJ-1*$^{-/-}$*SOD2*$^{+/-}$ mice by crossing *Parkin*$^{-/-}$*DJ-1*$^{-/-}$ mice with heterozygous SOD2 knockout mice because homozygous SOD2 deficiency is perinatal lethal (Li et al., 1995) while heterozygous SOD2 knockout mice have approximately 50% decrease in SOD2 activity and increased oxidative damage (Williams et al., 1998, Van Remmen et al., 1999). The triple mutant mice did not exhibit age-dependent nigral neuron loss, dopamine depletion, or motor behaviors characteristic of PD. Surprisingly, *Parkin*$^{-/-}$*DJ-1*$^{-/-}$*SOD1*$^{-/-}$ triple mutant mice had increased striatal dopamine levels and *Parkin*$^{-/-}$*DJ-1*$^{-/-}$ mice showed improved rotarod performance. Our data demonstrate that superoxide dismutase is not critical for preventing nigral cell loss caused by Parkin and DJ-1 deficiencies. The increase in striatal dopamine in *Parkin*$^{-/-}$*DJ-1*$^{-/-}$*SOD1*$^{-/-}$ mice indicates

significant abnormalities within the nigrostriatal pathway. These surprising behavioral and neurotransmitter abnormalities suggest that the pathogenic mechanisms of *Parkin* and *DJ-1* mutations may involve dysregulation of nigrostriatal dopaminergic neurotransmission.

Additional Material and Methods

Grip strength

Mice were suspended by the tail and allowed to clasp a grip bar with both forelimbs. Gentle constant horizontal resistance was applied until the mouse released the bar. Force was measured in grams by a force transducer attached to the bar. The greatest three of five trials per mouse were averaged.

Wheel running

We used a voluntary wheel running paradigm to analyze the locomotor activity of wild type and *Parkin*$^{-/-}$*DJ-1*$^{-/-}$ mice over 2-week period. We also used a light/dark paradigm in addition to the wheel running to investigate any changes in circadian rythms in the mutant mice. Mice were placed in individual cages with running wheels attached to a sensor that recorded revolutions for 2 weeks. Mice were on a normal 12 hour light/dark cycle.

Tritiated Dopamine Ligand Binding to D1 and D2-like Receptors in the Striatum

The rostral half of unfixed mouse brains were sectioned in the coronal plane on a cryostat at a thickness of 20 microns. Slides were preincubated in assay buffer (50 mM Tris, 120 mM sodium chloride, 5 mM potassium chloride, 1 mM magnesium chloride, 40 nM ketanserin)

for 20 min at room temperature. Slides were then incubated in buffer containing either 2 nM tritiated SCH 23390 (PerkinElmer, Boston, MA) or 5 nM tritiated spiperone (PerkinElmer, Boston, MA). Cold competition of tritiated siperone with 10μm cold siperone, or of tritiated SCH 23390 with 1μm cold SCH 23390 was used to assess non-specific signal. Following a 1-hour incubation, slides were washed twice in ice-cold buffer and then rinsed in cold water prior to incubation overnight at 4C in a desiccator with paraformaldehyde powder in the bottom to fix the tissue without washing away the ligand. Slides were dried for 2 hours in a desiccator with Dri-Rite and exposed to Kodak BioMax MS film with the Kodak BioMax TranScreen-LE Intensifying Screen for seven days (D1) or 5-7 weeks (D2). Films were analyzed for both density and area of binding using Adobe Photoshop software. Measurements from twenty sections per animal were averaged prior to statistical analysis. Four mice per genotype were included in the analysis.

Hematoxylin and eosin staining (H&E)

Muscle tissue was taken from the extensor digitorum longus (EDL) and soleus (SOL) muscles and frozen sections were incubated 45 minutes in hematoxylin stain followed by a water rinse, then a 0.3% hydrochloric acid in ethanol rinse. Slides were incubated in eosin for 2 minutes, dehydrated and mounted for imaging with a light microscope, as above.

Muscle fiber typing

Extensor digitorum longus (EDL) and soleus (SOL) muscles were dissected from wild type and *Parkin*$^{-/-}$*DJ-1*$^{-/-}$ mice and flash frozen. Muscles were cut into thin sections and

maintained at -20°C. Sections were stained with metachromatic ATPase as previously published (Ogilvie 1990). Briefly, sections were pre-incubated with ATPase 8 minutes (pH 4.5), rinsed twice for 3 minutes in Tris buffer (pH 7.8), then incubated with ATP (pH9.4) for 25 minutes at room temperature. Slides were rinsed 3 times with calcium chloride and counterstained with Toluene blue 0.1% for 1 minute, cleared in ethanol, then xylene, and mounted for imaging with a light microscope, as above.

Results

No neuronal loss in the substantia nigra of mutant mice

Triple knockout mice bearing combined loss-of-function mutations in the PD-linked genes *Parkin* and *DJ-1*, as well as the antioxidants *SOD1 or SOD2*, were born at the expected Mendelian ratio and had no apparent differences in viability compared to wild type mice. Because the pathological hallmark of PD is the progressive loss of dopamine neurons in the SN, we postulated that mice with a deficiency for Parkin, DJ-1 and major antioxidant proteins would show an age-dependent loss of dopamine neurons in the SN and thereby model human PD neuropathology. We used rigorous stereology to obtain unbiased estimates of the number of dopaminergic neurons, marked by immunohistochemical staining for tyrosine hydroxylase (TH), in coronal paraffin sections of wild type and mutant mice. We analyzed cohorts of mice at ages 7, 16 and 18 months. There was no statistically significant difference between the wild type mice and any of the mutant mice at any age by ANOVA (Figure 1). These data indicate that the number of nigral dopaminergic neurons is not significantly altered in *Parkin*$^{-/-}$*DJ-1*$^{-/-}$*SOD1*$^{-/-}$ mice or *Parkin*$^{-/-}$*DJ-1*$^{-/-}$*SOD2*$^{+/-}$ mice compared

to wild type mice at young or old ages.

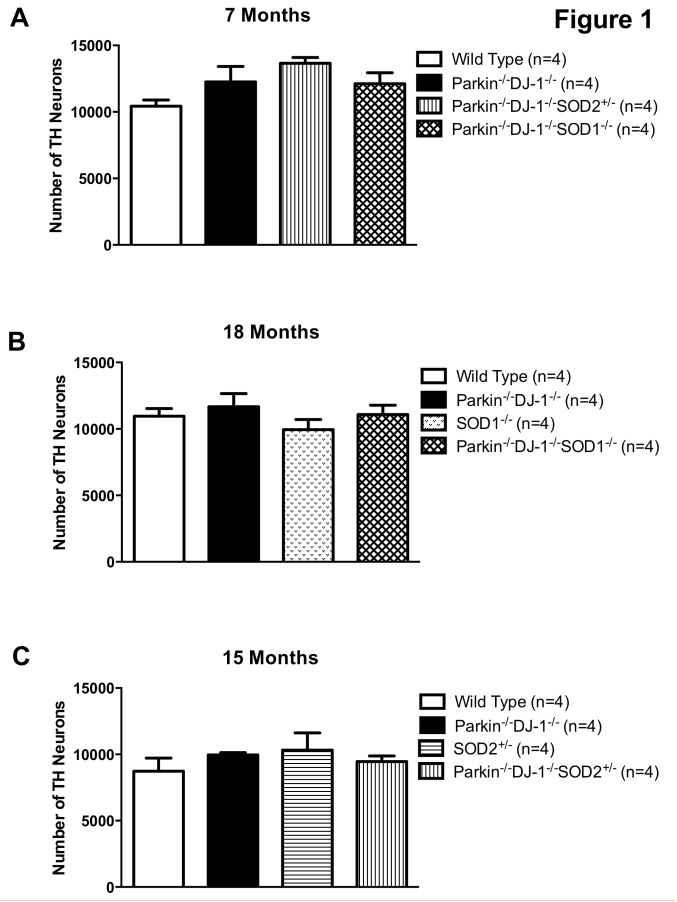

Figure A-1.No changes in the total bilateral number of TH-positive nigral neurons estimated by rigorous stereology. Dopaminergic neurons of separate cohorts of mice were counted for 7 month old mice (A) and 18 month old mice (B) and 16 month old mice (C). n = 4 for all genotypes. One-way ANOVA showed no differences between any of the genotypes ($p \geq 0.1$).

Dopamine levels are elevated in the striatum of triple mutant mice

Although we observed no change in dopaminergic nigral neurons, loss of dopaminergic terminals in the striatum may precede loss of cell bodies in the SN. Therefore, we used HPLC with electrochemical detection to measure levels of dopamine, serotonin and their metabolites in the striatum of wild type and mutant mice. Surprisingly, we found a consistent and significant increase in dopamine in the striatum of *Parkin$^{-/-}$DJ-1$^{-/-}$SOD1$^{-/-}$* mice, but not in *Parkin$^{-/-}$DJ-1$^{-/-}$SOD2$^{+/-}$,* compared to wild type mice (Figure 2).

In a younger cohort of mice, aged 7 months, levels of dopamine in the striatum of *Parkin*$^{-/-}$ *DJ-1*$^{-/-}$*SOD1*$^{-/-}$ mice were significantly higher compared to wild type mice ($p<0.001$; Figure 2A). *Parkin*$^{-/-}$*DJ-1*$^{-/-}$*SOD1*$^{-/-}$ mice had significantly increased dopamine turnover and significantly higher striatal levels of serotonin (5-HT), the serotonin metabolite 5-hydroxyindoleacetic acid (5-HIAA) and the dopamine metabolite homovanillic acid (HVA) compared to wild type mice ($p<0.05$). *Parkin*$^{-/-}$*DJ-1*$^{-/-}$*SOD2*$^{+/-}$ mice had significantly higher levels of the dopamine metabolites 3-methoxytyramine (3-MT) and HVA compared to wild type mice ($p<0.05$). There were no significant differences between wild type mice and *Parkin*$^{-/-}$*DJ-1*$^{-/-}$ mice in the levels of striatal dopamine, serotonin or any metabolites (data not shown).

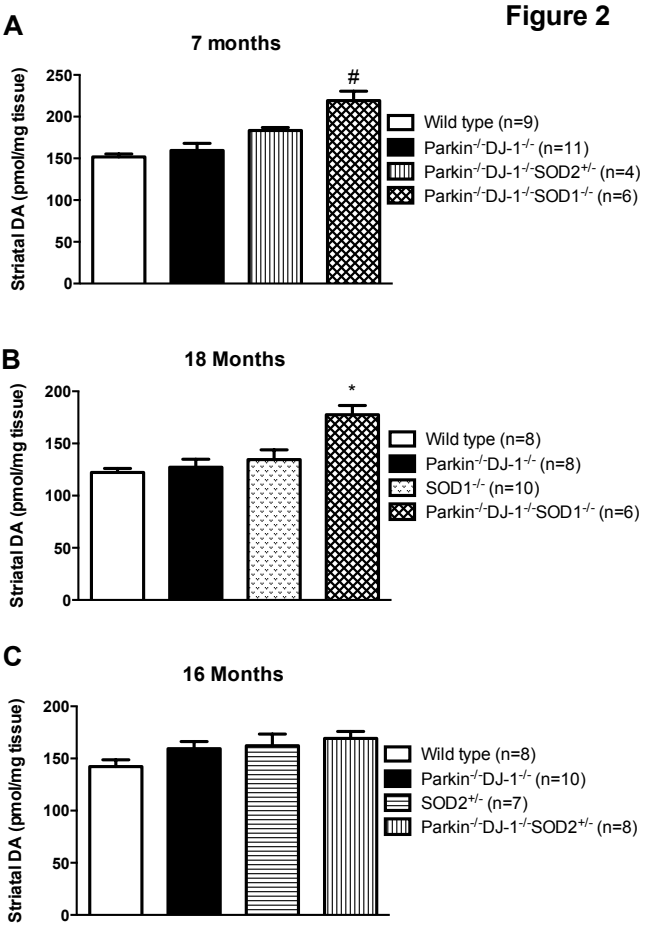

Figure A-2. Dopamine levels are elevated in *Parkin*$^{-/-}$*DJ-1*$^{-/-}$*SOD1*$^{-/-}$ mice. Total dopamine levels were measured by HPLC analysis in separate cohorts of mice ages 7 (A), 18 (B) and 16 (C) months ($n \geq 6$). (*$p \leq 0.001$ by one way ANOVA; #$p<0.001$ by Kruskal-Wallis one way ANOVA on ranks)

In an 18 month cohort of wild type, *Parkin*$^{-/-}$*DJ-1*$^{-/-}$*, SOD1*$^{-/-}$, and *Parkin*$^{-/-}$*DJ-1*$^{-/-}$*SOD1*$^{-/-}$ mice, there was a significant increase in striatal dopamine *Parkin*$^{-/-}$*DJ-1*$^{-/-}$*SOD1*$^{-/-}$ mice compared to wild type mice ($p < 0.001$) (Figure 2B). Striatal serotonin levels were significantly increased in *Parkin*$^{-/-}$*DJ-1*$^{-/-}$*SOD1*$^{-/-}$ mice compared to wild type mice ($p<0.001$). *Parkin*$^{-/-}$*DJ-1*$^{-/-}$*SOD1*$^{-/-}$ mice also had significantly higher levels of the dopamine metabolites dihydroxyphenylacetic acid (DOPAC) and HVA compared to wild type mice ($p<0.05$). There were no significant differences between genotypes in dopamine turnover or serotonin turnover.

Analysis of a cohort of 16 month old wild type, *SOD2*$^{+/-}$, *Parkin*$^{-/-}$*DJ-1*$^{-/-}$, and *Parkin*$^{-/-}$*DJ-1*$^{-/-}$ *SOD2*$^{+/-}$ mice showed a trend toward an increase in striatal dopamine in *Parkin*$^{-/-}$*DJ-1*$^{-/-}$ *SOD2*$^{+/-}$ mice compared to wild type mice ($p=0.101$) (Figure 2C). Striatal serotonin levels were significantly increased in *Parkin*$^{-/-}$*DJ-1*$^{-/-}$*SOD2*$^{+/-}$ mice compared to wild type mice ($p<0.05$). Also HVA and 3-MT were increased in *Parkin*$^{-/-}$*DJ-1*$^{-/-}$ and *Parkin*$^{-/-}$*DJ-1*$^{-/-}$ *SOD2*$^{+/-}$ mice compared to wild type mice ($p<0.05$). There were no significant differences between genotypes in dopamine turnover or serotonin turnover.

D1 and D2-like receptor densities in the striatum of mutant mice are similar to wild type

The significant increase in dopamine levels in triple mutant mice prompted us to measure levels of dopamine receptors in the striatum. We expected to see a compensatory change in dopamine receptor density in response to elevated dopamine levels in *Parkin*$^{-/-}$*DJ-1*$^{-/-}$*SOD1*$^{-/-}$ mice compared to wild type mice. Using radiolabeled dopamine receptor ligands, we quantified the abundance of D1-like and D2-like dopamine receptors in the striatum in fresh frozen sections. Contrary to the expected results, we saw no change in the binding of radiolabeled ligands to D1- or D2-type dopamine receptors ($p > 0.4$) (Figure 3).

Figure 3

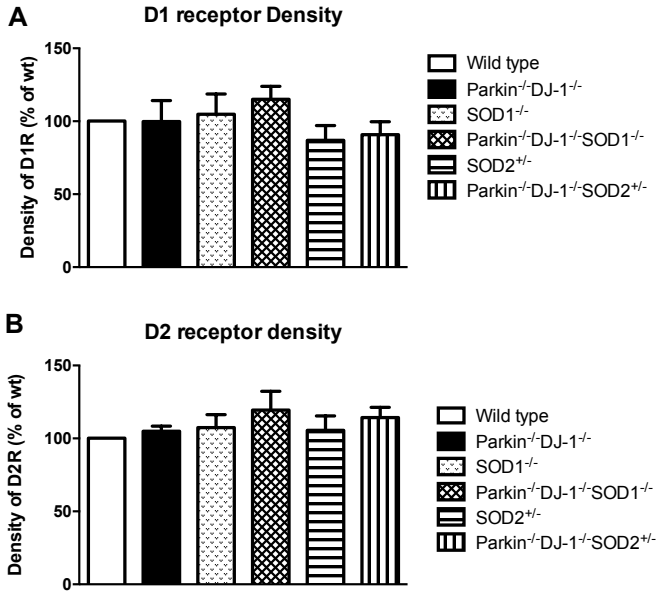

Figure A-3. Density of D1 and D2 receptors is unchanged in mutant mice. Striatal D1 and D2 dopamine receptor density was measured by radioligand binding to coronal sections through the striatum ($n \geq 3$ animals per genotype). One-way ANOVA showed no differences between genotypes in the density of D1 or D2-type dopamine receptors.

Locomotor behavior is unchanged in mutant mice

Because Parkinson's disease causes deficits in motor function, we investigated whether mice with combined PD-linked mutations have altered performance in established behavioral tests of motor function. The locomotor test measures total activity, and is used to identify mice that are hypoactive or hyperactive compared to wild type mice. We hypothesized that mice deficient for Parkin, DJ-1 and antioxidants would have an age-dependent locomotor deficit, manifested as reduced ambulatory behavior compared to age matched controls. A 7-month old cohort of wild type, *Parkin$^{-/-}$DJ-1$^{-/-}$*, *Parkin$^{-/-}$DJ-1$^{-/-}$SOD2$^{+/-}$*, and *Parkin$^{-/-}$DJ-1$^{-/-}$SOD1$^{-/-}$* mice exhibited no locomotor deficits ($p = 0.126$) (Figure 4A). The activity of all experimental groups was affected by time in the apparatus, indicating that all mice acclimated to the novel environment over the course of two hours, as indicated by a gradual decrease in the number of beam breaks.

Figure 4

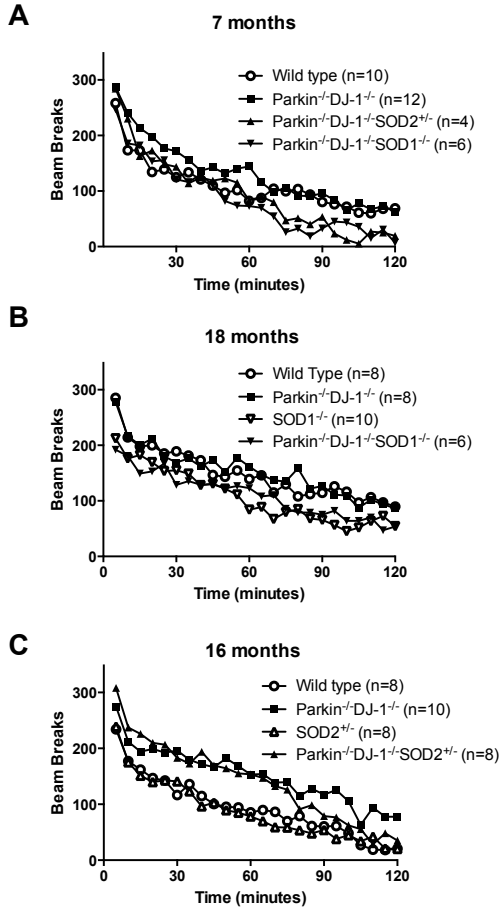

Figure A-4. Locomotor behavior is unchanged. Separate cohorts of mice were tested at 7 (A), 18 (B), and 16 (C) months. All cohorts of mice have an initial increase upon introduction to a new environment followed by decreased activity as the mice habituate. No significant differences are seen between genotypes in (A) and (B) by repeated measures ANOVA. There is a main effect of genotype in (C) ($p = 0.005$). Tukey post-hoc comparison shows a significant difference between *Parkin*$^{-/-}$*DJ-1*$^{-/-}$ mice and wild type ($p = 0.031$), and between *Parkin*$^{-/-}$*DJ-1*$^{-/-}$ and *SOD2*$^{+/-}$ ($p = 0.015$).

We assessed voluntary locomotion in an 18 month old cohort wild type, *Parkin*$^{-/-}$*DJ-1*$^{-/-}$, *SOD1*$^{-/-}$, and *Parkin*$^{-/-}$*DJ-1*$^{-/-}$*SOD1*$^{-/-}$ mice (Figure 4B). There was no difference in locomotor activity between any of the genotypes tested ($p = 0.152$). Analysis of locomotor activity in a cohort of 16 month old wild type, *Parkin*$^{-/-}$*DJ-1*$^{-/-}$, *SOD2*$^{+/-}$, and *Parkin*$^{-/-}$*DJ-1*$^{-/-}$*SOD2*$^{+/-}$ mice showed a main effect of genotype ($p = 0.005$) (Figure 4C). Further analysis (Tukey test) revealed a significant difference between *Parkin*$^{-/-}$*DJ-1*$^{-/-}$ mice and wild type ($p = 0.031$) and between *Parkin*$^{-/-}$*DJ-1*$^{-/-}$ and *SOD2*$^{+/-}$ ($p = 0.015$). Mice deficient for *SOD2* were not different from wild type in this experiment, nor were double mutant mice different from triple mutant

mice.

Figure 5

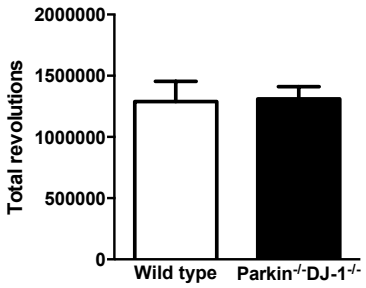

Figure A-5. *Parkin*$^{-/-}$*DJ-1*$^{-/-}$ perform similarly to wild type on the wheel running test. Five-month old *Parkin*$^{-/-}$*DJ-1*$^{-/-}$ and wild type mice were housed individually in cages equipped with running wheels. Total wheel revolutions during 2 weeks were recorded and averaged. n= 9 wild type and n= 8 *Parkin*$^{-/-}$*DJ-1*$^{-/-}$ mice. No significant difference was observed between genotypes by student's t-test.

Because the locomotor test shows an initial hyperactivity due to the novelty of the environment, we sought to measure voluntary locomotion over an extended period of time. We used a wheel running paradigm to compare the home cage locomotor activity of individually housed five-month old wild type and *Parkin*$^{-/-}$*DJ-1*$^{-/-}$ mice over a period of two weeks. There was no significant difference between genotypes in the total wheel revolutions according to student's t-test ($p=0.855$) (Figure 5). This indicates that there are no abnormalities in voluntary home cage locomotor activity in *Parkin*$^{-/-}$*DJ-1*$^{-/-}$ mice compared to wild type mice.

Rotarod behavior is altered in mice with combined mutations linked to PD

The rotorod task is commonly used to measure the ability of a rodent to sustain complex coordinated movement over time and has been used as a measure of basal ganglia function

and as a functional measure of neurodegeneration in mouse models of amyotrophic lateral sclerosis (ALS), Huntington's disease (HD) and other neurodegenerative diseases (Ramaswamy et al., 2009). No abnormal rotorod phenotypes have been reported for *Parkin*$^{-/-}$, *DJ-1*$^{-/-}$, *SOD1*$^{-/-}$, or *SOD2*$^{+/-}$ mice (Goldberg et al., 2003, Von Coelln et al., 2004, Chen et al., 2005, Goldberg et al., 2005, Sato et al., 2006, Chandran et al., 2008, Perucho et al., 2010, Thomas et al., 2011). We assessed the rotorod performance of wild type, *Parkin*$^{-/-}$*DJ-1*$^{-/-}$, *SOD1*$^{-/-}$, *SOD2*$^{+/-}$, *Parkin*$^{-/-}$*DJ-1*$^{-/-}$*SOD1*$^{-/-}$, and *Parkin*$^{-/-}$*DJ-1*$^{-/-}$*SOD2*$^{+/-}$ at various ages (Figure 6). As expected, there was a main effect of trial for each experiment, indicating that there is a learning process associated with the task and all groups improve over the course of the 8 trials. There was also a main effect of genotype in each experiment, but surprisingly, the *Parkin*$^{-/-}$*DJ-1*$^{-/-}$ mice had an increased latency to fall instead of the expected decreased latency, compared to wild type mice.

We first tested a cohort of 6 month old wild type, *Parkin*$^{-/-}$*DJ-1*$^{-/-}$, and the two triple mutant lines: *Parkin*$^{-/-}$*DJ-1*$^{-/-}$*SOD1*$^{-/-}$ and *Parkin*$^{-/-}$*DJ-1*$^{-/-}$*SOD2*$^{+/-}$. Two-way repeated measures ANOVA showed that both number of trials ($p < 0.001$) and genotype ($p < 0.001$) affected the outcomes (Figure 6A) and was true for all analyses (Figure 6A-D). Post-hoc analysis revealed a significant and surprising difference between wild type mice and both *Parkin*$^{-/-}$*DJ-1*$^{-/-}$ and *Parkin*$^{-/-}$*DJ-1*$^{-/-}$*SOD2*$^{+/-}$ mice ($p < 0.001$), with the mutant mice performing better than wild type (Figure 6A). In a 9 month old cohort of wild type, *Parkin*$^{-/-}$*DJ-1*$^{-/-}$, and *SOD2*$^{+/-}$ mice, we found a main effect of genotype ($p < 0.001$) and trial ($p < 0.001$) (Figure 6B). Post-hoc analysis showed that the *Parkin*$^{-/-}$*DJ-1*$^{-/-}$ mice again had increased latency to fall

compared to wild type mice ($p < 0.001$).

We analyzed two cohorts of aged wild type, *Parkin*$^{-/-}$*DJ-1*$^{-/-}$, and either *Parkin*$^{-/-}$*DJ-1*$^{-/-}$*SOD1*$^{-/-}$ or *Parkin*$^{-/-}$*DJ-1*$^{-/-}$*SOD2*$^{+/-}$ mice with *SOD1*$^{-/-}$ or *SOD2*$^{+/-}$ mice as additional controls. In a 17-month old cohort of wild type, *Parkin*$^{-/-}$*DJ-1*$^{-/-}$*SOD1*$^{-/-}$, *SOD1*$^{-/-}$ mice and *Parkin*$^{-/-}$*DJ-1*$^{-/-}$ *SOD1*$^{-/-}$ mice (Figure 6C), pair-wise comparisons showed that *Parkin*$^{-/-}$*DJ-1*$^{-/-}$ mice stayed on the rod significantly longer than wild type mice ($p = 0.05$), which stayed on the rod significantly longer than *Parkin*$^{-/-}$*DJ-1*$^{-/-}$*SOD1*$^{-/-}$ mice ($p = 0.03$). Observation of home cage behavior and handling indicated that the locomotor ability of *SOD1*$^{-/-}$ mice deteriorates with age. The *Parkin*$^{-/-}$*DJ-1*$^{-/-}$*SOD1*$^{-/-}$ mice are lethargic and weak by age 17 months, similar to the *SOD1*$^{-/-}$ mice. The loss of the SOD1 protein apparently causes poor coordination or stamina, resulting in decreased rotarod latencies for *SOD1*$^{-/-}$ mice and *Parkin*$^{-/-}$*DJ-1*$^{-/-}$*SOD1*$^{-/-}$ mice compared to wild type mice.

In a 15-month-old cohort of *Parkin*$^{-/-}$*DJ-1*$^{-/-}$*SOD2*$^{+/-}$ mice, *SOD2*$^{+/-}$ mice, *Parkin*$^{-/-}$*DJ-1*$^{-/-}$ mice and wild type mice, 2-way repeated measures ANOVA showed a main effect of genotype ($p < 0.001$) and trial ($p < 0.001$) (Figure 6D). Pair-wise comparisons show that *Parkin*$^{-/-}$*DJ-1*$^{-/-}$ mice stayed on the rotarod significantly longer than wild type mice ($p < 0.001$) and *SOD2*$^{+/-}$ mice ($p < 0.001$; Figure 6D). Likewise, *Parkin*$^{-/-}$*DJ-1*$^{-/-}$*SOD2*$^{+/-}$ mice had increased latency to fall compared to wild type mice ($p < 0.001$) and *SOD2*$^{+/-}$ mice ($p < 0.001$). There was no significant difference between the rotarod performance of *Parkin*$^{-/-}$*DJ-1*$^{-/-}$ mice and *Parkin*$^{-/-}$*DJ-1*$^{-/-}$*SOD2*$^{+/-}$ mice. The rotarod latencies of wild type mice and

SOD2$^{+/-}$ mice were also similar, indicating that the loss of one allele of *SOD2* did not affect rotarod performance.

Figure 6

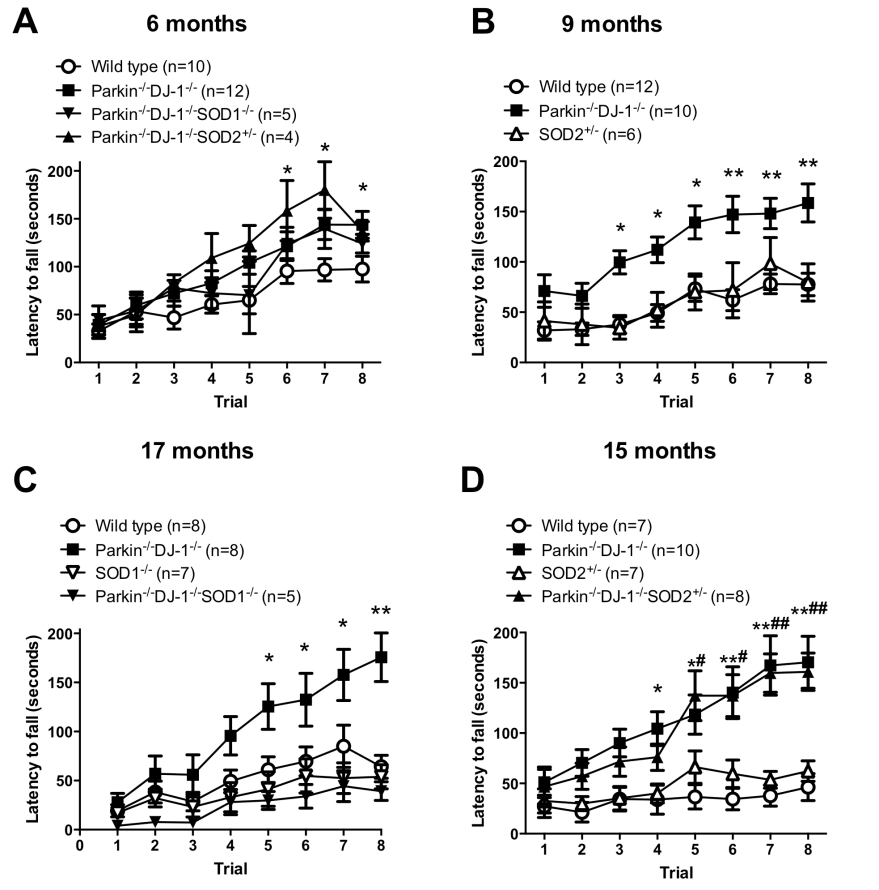

Figure A-6. *Parkin*$^{-/-}$*DJ-1*$^{-/-}$ and aged *Parkin*$^{-/-}$*DJ-1*$^{-/-}$*SOD2*$^{+/-}$ mice have improved rotarod performance. Latency to fall from an accelerating rotarod was measured for eight trials of five minutes over two days. Separate cohorts of mice were tested at 6 (A), 9 (B), 17 (C) and 15 (D) months. A minimum of 4 mice were tested per genotype per cohort. Symbols represent the mean ± SEM time (seconds) before falling off the rod for each of 8 trials. All genotypes learned the task over the course of multiple trials, however, *Parkin*$^{-/-}$*DJ-1*$^{-/-}$ mice had increased latency to fall compared to wild type. (*$p<0.01$, **$p<0.001$ by 2-way repeated measures ANOVA).

The unexpected increase in rotorod performance of *Parkin*$^{-/-}$*DJ-1*$^{-/-}$ mice prompted us to determine whether these mice have increased ability to grip the rotating rod, thus facilitating their ability to remain on the rod for longer periods of time. We analyzed the grip strength of a cohort of 6-month old wild type, *Parkin*$^{-/-}$*DJ-1*$^{-/-}$, *Parkin*$^{-/-}$*DJ-1*$^{-/-}$*SOD1*$^{-/-}$, and *Parkin*$^{-/-}$*DJ-1*$^{-/-}$*SOD2*$^{+/-}$ mice. Although there was a trend towards reduced grip strength in the mutant mice, there was no difference between genotypes (p=0.352) (Figure 7A). Therefore the improved rotorod performance of the double and triple mutant mice was not due to increased grip strength.

Other characteristics that could impact the striking differences observed in rotarod behavior are differences in reflexes or sensory functions of the mice. This prompted us to measure acoustic startle response behavior in the mice using the acoustic startle test, which measures the innate reflex of the mouse in response to a sudden and unexpected acute stimulus, in this case a loud noise. Analysis of a cohort of 7 month old wild type, *Parkin*$^{-/-}$*DJ-1*$^{-/-}$, *Parkin*$^{-/-}$*DJ-1*$^{-/-}$*SOD1*$^{-/-}$, and *Parkin*$^{-/-}$*DJ-1*$^{-/-}$*SOD2*$^{+/-}$ mice, showed a main effect of genotype ($p < 0.001$) and stimulus intensity ($p < 0.001$). Post-hoc comparison revealed a significant decrease in startle response in *Parkin*$^{-/-}$*DJ-1*$^{-/-}$ ($p < 0.001$) and *Parkin*$^{-/-}$*DJ-1*$^{-/-}$*SOD1*$^{-/-}$ ($p < 0.001$) compared to wild type (Figure 7B). There was a trend toward reduced startle response in *Parkin*$^{-/-}$*DJ-1*$^{-/-}$*SOD2*$^{+/-}$ mice compared to wild type mice, but this was not significant, perhaps due to the smaller sample size of this genotype. Decreased startle responses in *Parkin*$^{-/-}$*DJ-1*$^{-/-}$ and *Parkin*$^{-/-}$*DJ-1*$^{-/-}$*SOD1*$^{-/-}$ indicate impairment of reflexes and/or sensory functions compared to wild type mice. However, this would not explain the observed

increased latency to fall in these genotypes

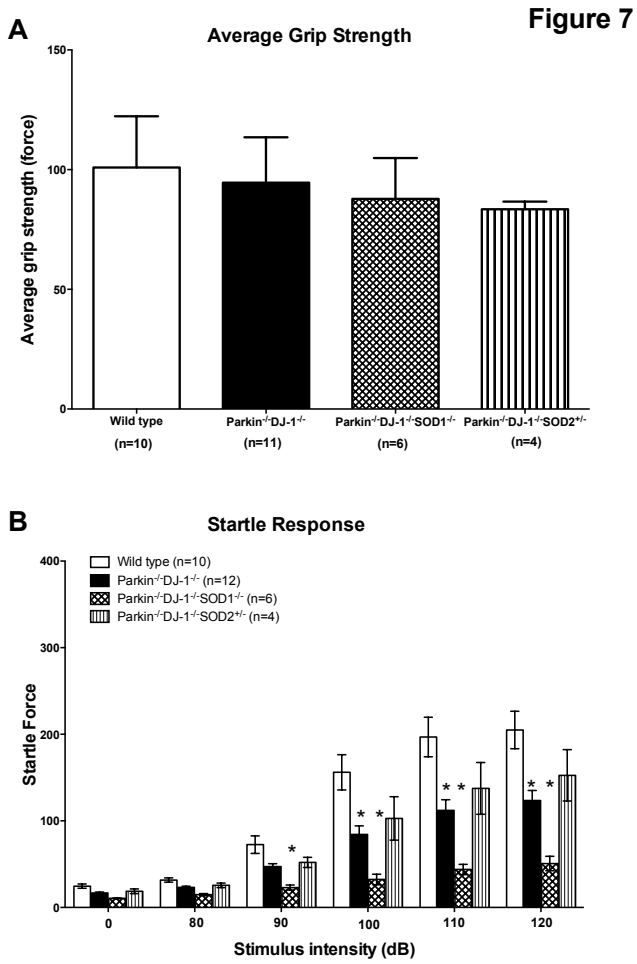

Figure A-7. Knockout mice have similar grip strength and decreased startle response compared to wild type. (A) 6 month old *Parkin*$^{-/-}$ *DJ-1*$^{-/-}$, *Parkin*$^{-/-}$*DJ-1*$^{-/-}$ *SOD1*$^{-/-}$ and *Parkin*$^{-/-}$*DJ-1*$^{-/-}$*SOD2*$^{+/-}$ mice had average grip strength statistically similar to wild type mice by 1 way ANOVA ($p = 0.07$). (B) The intensity of the response to an audible stimulus was measured in a cohort of 7 month old mice (n = 4-12 mice per genotype). (*$p < 0.0001$ by 2 way ANOVA).

Because decreased startle amplitudes could indicate decreased anxiety levels and because fear of falling off the rotarod apparatus could contribute to rotarod behavior performance, we tested wild type and mutant mice for changes in fear or anxiety using several established behavioral paradigms including the light/dark test, the open field test and the elevated plus maze. We did not observe any difference between genotypes in any of these behavior tests (data not shown). Therefore, the increased rotorod performance of the double and triple mutant mice does not appear to be due to altered anxiety.

We also conducted the forced swim test as part of our comprehensive battery of behavioral tests in an effort to better characterize these mice and possibly explain the altered rotarod performance. The forced swim test (FST) is a test of forced motor behavior developed to predict the efficacy of antidepressant drugs (Porsolt, Le Pichon et al. 1977) and is particularly sensitive to compounds acting on the serotonin and norepinephrine systems. In a cohort of wild type and mutant mice, average age 6 months, we found a main effect of genotype ($p = 0.006$) (Figure 8A). Further analysis showed that immobility time was significantly less in *Parkin*$^{-/-}$*DJ-1*$^{-/-}$ mice ($p = 0.005$) and *Parkin*$^{-/-}$*DJ-1*$^{-/-}$*SOD2*$^{+/-}$ mice ($p = 0.027$) compared to wild type. Post hoc analysis of a second 14 month old cohort of wild type, *Parkin*$^{-/-}$, and *Parkin*$^{-/-}$*DJ-1*$^{-/-}$ demonstrated a significant decrease in immobility time *Parkin*$^{-/-}$*DJ-1*$^{-/-}$ mice compared to wild type ($p < 0.001$). However, there was no difference between *Parkin*$^{-/-}$ and wild type mice, indicating that loss of Parkin alone is not sufficient for the manifestation of the FST phenotype (Figure 8B). A test of 16 month old wild type, *DJ-1*$^{-/-}$ and *Parkin*$^{-/-}$*DJ-1*$^{-/-}$ mice, both *DJ-1*$^{-/-}$ mice and *Parkin*$^{-/-}$*DJ-1*$^{-/-}$ mice showed decreased immobility time compared to wild type ($p < 0.001$) (Figure 8C). Together these data indicate that the loss of DJ-1 alone is sufficient to confer the increased mobility observed in the forced swim test.

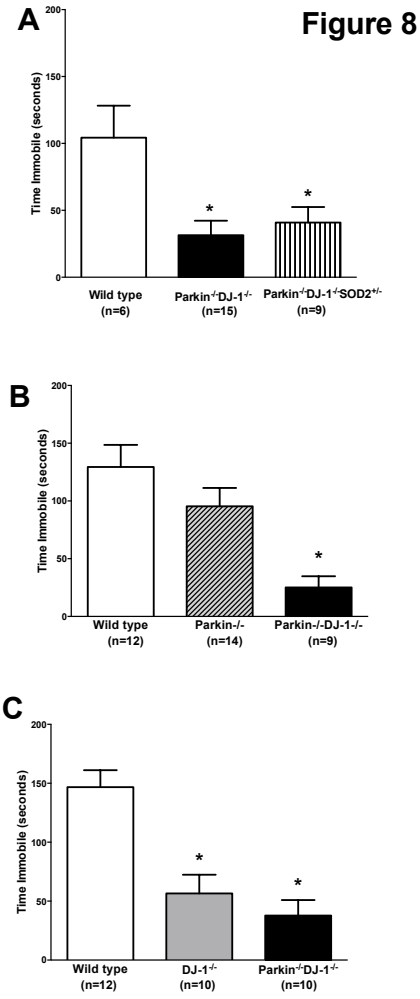

Figure A-8. *DJ-1*$^{-/-}$, *Parkin*$^{-/-}$ *DJ-1*$^{-/-}$ and *Parkin*$^{-/-}$*DJ-1*$^{-/-}$ *SOD2*$^{+/-}$ mice have decreased time immobile in the Porsolt forced swim test. Separate cohorts of mice were tested at age 6 months with a minimum n = 6. (*$p \leq 0.01$ by 1-way ANOVA).

Muscle, heart, and cerebellar histology appear normal in mice with combined mutations linked to PD

Parkin is highly expressed in the heart and muscle tissue, as well as most areas of the brain (Kitada et al., 1998). Additionally, the ability to stay on the rotorod is highly dependent on cerebellar function. Therefore, histology of muscle, heart and cerebellum was performed in order to test for abnormalities that might account for enhanced rotarod performance in the *Parkin*$^{-/-}$*DJ-1*$^{-/-}$ mice. Hematoxylin and eosin staining of paraffin sections of hearts from 18 month old wild type and *Parkin*$^{-/-}$*DJ-1*$^{-/-}$ mice showed normal organ structure as well as

normal cellular appearance of heart muscle in mice of both genotypes (data not shown). Hematoxylin and eosin staining of coronal brain sections showed normal anatomical and cellular organization of the cerebellum (data not shown). We hypothesized we would see a difference in the ratio of fast twitch to slow twitch muscle fiber types, providing the *Parkin*$^{-/-}$ *DJ-1*$^{-/-}$ mice fatigue-resistant muscles and better stamina on the rotorod. The ratio and morphology of type I and type II muscle fiber types appeared similar in metachromatic ATPase stained soleus muscle from *Parkin*$^{-/-}$*DJ-1*$^{-/-}$ mice compared to wild type mice (data not shown).

Discussion

Despite years of effort by many researchers generating and characterizing mice with recessive mutations in Parkin and DJ-1 linked to familial PD, no Parkin or DJ-1 knockout mouse line exhibits all, or even most, of the key clinical and neuropathological features of PD (Goldberg et al., 2003, Itier et al., 2003, Palacino et al., 2004, Von Coelln et al., 2004, Fleming et al., 2005, Goldberg et al., 2005, Kim et al., 2005, Perez et al., 2005, Perez and Palmiter, 2005, Fleming and Chesselet, 2006, Sato et al., 2006, Andres-Mateos et al., 2007). This contrasts with mouse models of other neurodegenerative diseases such as Huntington's disease and amyotrophic lateral sclerosis that reproduce the major neuropathological and behavioral features of these diseases, including delayed onset of motor behavior deficits as well as selective and progressive neurodegeneration and protein inclusions (Joyce et al., 2011, Brooks et al., 2012). It is possible that compensatory changes protect mice from the neurodegeneration caused by Parkin and DJ-1 mutations or that additional stress is required

to initiate neurodegeneration in Parkin and DJ-1 knockout mice. Indeed, we have shown that Parkin knockout mice are more susceptible to nigral cell loss induced by chronic lipopolysaccharide exposure (Frank-Cannon et al., 2008) and others have shown that DJ-1 knockout mice are more susceptible to MPTP-induced nigral cell loss (Kim et al., 2005). Here we sought to generate better mouse models of progressive nigral cell loss induced by genetic mutations alone. We combined PD-linked mutations in Parkin and DJ-1 with deficiency for antioxidant enzymes that have been shown to be key determinants of susceptibility to nigrostriatal degeneration in mice (Andrews et al., 1996, Asanuma et al., 1998, Hirata et al., 1998, Ihara et al., 1999, Andreassen et al., 2001, Kunikowska and Jenner, 2003, Liang and Patel, 2004, Callio et al., 2005, Barkats et al., 2006, Wang et al., 2006, Tripanichkul et al., 2007, Boll et al., 2008, Lenzken et al., 2011, Wang et al., 2011b, Belluzzi et al., 2012, Sun et al., 2012).

Because *SOD1*$^{-/-}$ mice and *SOD2*$^{+/-}$ mice have also been shown to be more susceptible to neurotoxin-mediated nigral cell loss, we hypothesized that combining Parkin and DJ-1 deficiency with SOD1 or SOD2 deficiency would result in a loss of dopaminergic neurons. Contrary to our expectations, neither *Parkin*$^{-/-}$*DJ-1*$^{-/-}$*SOD1*$^{-/-}$ mice nor *Parkin*$^{-/-}$*DJ-1*$^{-/-}$*SOD2*$^{+/-}$ mice showed age-dependent loss of nigral dopamine neurons compared to wild type mice (Figure 1). It has been suggested that death of nigral dopamine neurons begins with the axon terminals in the striatum, from which a die-back process occurs, ultimately resulting in the loss of nigral neurons after loss of terminals has occurred (Bernheimer et al., 1973, Cheng et al., 2011, Pham et al., 2012). For this reason, we also measured the levels of dopamine and

its metabolites in the striatum (Figure 2). Surprisingly, *Parkin*$^{-/-}$*DJ-1*$^{-/-}$*SOD1*$^{-/-}$ mice but not *Parkin*$^{-/-}$*DJ-1*$^{-/-}$ mice or *SOD1*$^{-/-}$ mice had significantly increased levels of dopamine in the striatum. This indicates that SOD1 deficiency perturbs the nigrostriatal pathway, but only in combination with mutations linked to PD. The increased striatal dopamine did not cause hyperactivity or other apparent behavioral abnormalities specific to *Parkin*$^{-/-}$*DJ-1*$^{-/-}$*SOD1*$^{-/-}$ mice. Dopamine levels are tightly regulated by presynaptic autoreceptors that signal the regulation of tyrosine hydroxylase to alter dopamine synthesis. Therefore, we speculate that the increased striatal dopamine levels indicate that *Parkin*$^{-/-}$*DJ-1*$^{-/-}$*SOD1*$^{-/-}$ mice either have dopamine autoreceptor signaling defects or that the increased dopamine compensates for other defects in dopaminergic signaling. It is possible that, in the absence of the compensatory increase in striatal dopamine, we would have detected behavioral phenotypes specifically in *Parkin*$^{-/-}$*DJ-1*$^{-/-}$*SOD1*$^{-/-}$ mice. Alternatively, the increase in dopamine may be indicative of a compensatory change that is occurring in the striatum that might ultimately lead to neuronal death, either due to overwhelming the compensatory mechanisms of the cell or due to neurotoxicity of dopamine itself (Ben-Shachar et al., 2004, Hattoria et al., 2009). The increased striatal dopamine also could be due to interactions between the three genes to affect release, trafficking, dopamine production, or degradation. Consistent with this possibility, Parkin and DJ-1 have been found in cell membranes and vesicles (Kubo et al., 2001, Usami et al., 2011) and *Parkin*$^{-/-}$ and *DJ-1*$^{-/-}$ mice have been reported to have altered dopamine release, reuptake and signaling (Kitada et al., 1998, Jiang et al., 2004, Goldberg et al., 2005, Jiang et al., 2012).

The increased striatal dopamine in *Parkin*$^{-/-}$*DJ-1*$^{-/-}$*SOD1*$^{-/-}$ mice is consistent with the increased striatal dopamine we identified in mice deficient for Parkin, DJ-1 and another antioxidant protein, glutathione peroxidase 1 (Hennis et al., 2013). The lack of a significant change in striatal dopamine levels in *Parkin*$^{-/-}$*DJ-1*$^{-/-}$*SOD2*$^{+/-}$ mice may be due to SOD2 heterozygosity not being sufficient to produce a full phenotype in these mice. SOD2 homozygous knockout mice are not viable, thus precluding analysis of *Parkin*$^{-/-}$*DJ-1*$^{-/-}$*SOD2*$^{-/-}$ mice.

The increased striatal dopamine levels cannot explain the rotarod and forced swim test behavioral phenotypes because we did not observe an increase in striatal dopamine in the DJ-1 single knockout mice (which show the forced swim test phenotype) or Parkin-DJ-1 double knockout mice (which show the improved rotarod performance phenotype). Therefore, the behavioral and the neurochemical phenotypes observed in these mice are likely independent of each other. Mice deficient for both Parkin and DJ-1 consistently stayed on the rotarod longer than wild type mice. This finding holds true in both the young and aged cohorts of mice, although the phenotype is more pronounced in aged mice (Figure 6). The rotarod behavioral phenotype is consistent with independent cohorts of *Parkin*$^{-/-}$*DJ-1*$^{-/-}$ mice that we studied in combination with glutathione peroxidase deficiency (Hennis et al., 2013), which suggested that the increased rotarod latency of *Parkin*$^{-/-}$*DJ-1*$^{-/-}$ mice may be due to decreased distraction from the task compared to wild type mice.

The decreased startle response in *Parkin*$^{-/-}$*DJ-1*$^{-/-}$ mice might be due to Parkin deficiency

alone because *Parkin*$^{-/-}$ mice have previously been shown to have decreased startle response (Von Coelln et al., 2004). The greater decrease in startle response in *Parkin*$^{-/-}$*DJ-1*$^{-/-}$*SOD1*$^{-/-}$ mice is likely due to a loss of hearing in mice deficient for SOD1. The cochleae of *SOD1*$^{-/-}$ mice have been shown to have severe spiral ganglion cell degeneration by 7 months of age (Keithley et al., 2005).

It remains possible that abnormalities in antioxidants such as SOD1 or SOD2 are involved in the development or progression of PD. However, our study demonstrates that deficiency for these antioxidants does not directly cause nigral cell loss or parkinsonian-like locomotor deficits in the context of mice with PD-linked mutations in Parkin and DJ-1. The increase in striatal dopamine resulting from the combination of SOD1, Parkin and DJ-1 deficiency might represent early-stage nigrostriatal dysfunction and these mice may be useful for efforts to develop neuroprotective therapies targeting early stage abnormalities in PD pathogenesis.

BIBLIOGRAPHY

Abbas N, Lucking CB, Ricard S, Durr A, Bonifati V, De Michele G, Bouley S, Vaughan JR, Gasser T, Marconi R, Broussolle E, Brefel-Courbon C, Harhangi BS, Oostra BA, Fabrizio E, Bohme GA, Pradier L, Wood NW, Filla A, Meco G, Denefle P, Agid Y, Brice A (A wide variety of mutations in the parkin gene are responsible for autosomal recessive parkinsonism in Europe. French Parkinson's Disease Genetics Study Group and the European Consortium on Genetic Susceptibility in Parkinson's Disease. Hum Mol Genet 8:567-574.1999).

Abou-Sleiman PM, Healy DG, Wood NW (Causes of Parkinson's disease: genetics of DJ-1. Cell Tissue Res 318:185-188.2004).

Alam ZI, Jenner A, Daniel SE, Lees AJ, Cairns N, Marsden CD, Jenner P, Halliwell B (Oxidative DNA damage in the parkinsonian brain: an apparent selective increase in 8-hydroxyguanine levels in substantia nigra. J Neurochem 69:1196-1203.1997).

Aleyasin H, Rousseaux MW, Marcogliese PC, Hewitt SJ, Irrcher I, Joselin AP, Parsanejad M, Kim RH, Rizzu P, Callaghan SM, Slack RS, Mak TW, Park DS (DJ-1 protects the nigrostriatal axis from the neurotoxin MPTP by modulation of the AKT pathway. Proc Natl Acad Sci U S A 107:3186-3191.2010).

Amende I, Kale A, McCue S, Glazier S, Morgan JP, Hampton TG (Gait dynamics in mouse models of Parkinson's disease and Huntington's disease. J Neuroeng Rehabil 2:20.2005).

Andreassen OA, Ferrante RJ, Dedeoglu A, Albers DW, Klivenyi P, Carlson EJ, Epstein CJ, Beal MF (Mice with a partial deficiency of manganese superoxide dismutase show increased vulnerability to the mitochondrial toxins malonate, 3-nitropropionic acid, and MPTP. Exp Neurol 167:189-195.2001).

Andres-Mateos E, Perier C, Zhang L, Blanchard-Fillion B, Greco TM, Thomas B, Ko HS, Sasaki M, Ischiropoulos H, Przedborski S, Dawson TM, Dawson VL (DJ-1 gene deletion reveals that DJ-1 is an atypical peroxiredoxin-like peroxidase. Proc Natl Acad Sci U S A 104:14807-14812.2007).

Andrews AM, Ladenheim B, Epstein CJ, Cadet JL, Murphy DL (Transgenic mice with high levels of superoxide dismutase activity are protected from the neurotoxic effects of 2'-NH2-MPTP on serotonergic and noradrenergic nerve terminals. Mol Pharmacol 50:1511-1519.1996).

Ariga H, Takahashi-Niki K, Kato I, Maita H, Niki T, Iguchi-Ariga SM (Neuroprotective function of DJ-1 in Parkinson's disease. Oxid Med Cell Longev 2013:683920.2013).

Asanuma M, Hirata H, Cadet JL (Attenuation of 6-hydroxydopamine-induced dopaminergic nigrostriatal lesions in superoxide dismutase transgenic mice. Neuroscience 85:907-917.1998).

Bademci G, Vance JM, Wang L (Tyrosine hydroxylase gene: another piece of the genetic puzzle of Parkinson's disease. CNS Neurol Disord Drug Targets 11:469-481.2012).

Bajt ML, Ho YS, Vonderfecht SL, Jaeschke H (Reactive oxygen as modulator of TNF and fas receptor-mediated apoptosis in vivo: studies with glutathione peroxidase-deficient mice. Antioxid Redox Signal 4:733-740.2002).
Bao L, Avshalumov MV, Patel JC, Lee CR, Miller EW, Chang CJ, Rice ME (Mitochondria are the source of hydrogen peroxide for dynamic brain-cell signaling. J Neurosci 29:9002-9010.2009).
Barkats M, Horellou P, Colin P, Millecamps S, Faucon-Biguet N, Mallet J (1-methyl-4-phenylpyridinium neurotoxicity is attenuated by adenoviral gene transfer of human Cu/Zn superoxide dismutase. J Neurosci Res 83:233-242.2006).
Bedard C, Wallman MJ, Pourcher E, Gould PV, Parent A, Parent M (Serotonin and dopamine striatal innervation in Parkinson's disease and Huntington's chorea. Parkinsonism Relat Disord 17:593-598.2011).
Belluzzi E, Bisaglia M, Lazzarini E, Tabares LC, Beltramini M, Bubacco L (Human SOD2 modification by dopamine quinones affects enzymatic activity by promoting its aggregation: possible implications for Parkinson's disease. PLoS One 7:e38026.2012).
Ben-Shachar D, Zuk R, Gazawi H, Ljubuncic P (Dopamine toxicity involves mitochondrial complex I inhibition: implications to dopamine-related neuropsychiatric disorders. Biochem Pharmacol 67:1965-1974.2004).
Benabid AL, Benazzouz A, Hoffmann D, Limousin P, Krack P, Pollak P (Long-term electrical inhibition of deep brain targets in movement disorders. Mov Disord 13 Suppl 3:119-125.1998).
Benabid AL, Chabardes S, Mitrofanis J, Pollak P (Deep brain stimulation of the subthalamic nucleus for the treatment of Parkinson's disease. Lancet Neurol 8:67-81.2009).
Bendor JT, Logan TP, Edwards RH (The Function of alpha-Synuclein. Neuron 79:1044-1066.2013).
Benzi G, Pastoris O, Marzatico F, Villa RF (Cerebral enzyme antioxidant system. Influence of aging and phosphatidylcholine. J Cereb Blood Flow Metab 9:373-380.1989).
Bernheimer H, Birkmayer W, Hornykiewicz O, Jellinger K, Seitelberger F (Brain dopamine and the syndromes of Parkinson and Huntington. Clinical, morphological and neurochemical correlations. J Neurol Sci 20:415-455.1973).
Betarbet R, Sherer TB, MacKenzie G, Garcia-Osuna M, Panov AV, Greenamyre JT (Chronic systemic pesticide exposure reproduces features of Parkinson's disease. Nat Neurosci 3:1301-1306.2000).
Bian M, Liu J, Hong X, Yu M, Huang Y, Sheng Z, Fei J, Huang F (Overexpression of parkin ameliorates dopaminergic neurodegeneration induced by 1- methyl-4-phenyl-1,2,3,6-tetrahydropyridine in mice. PLoS One 7:e39953.2012).
Blandini F (Neural and immune mechanisms in the pathogenesis of Parkinson's disease. J Neuroimmune Pharmacol 8:189-201.2013).
Boll MC, Alcaraz-Zubeldia M, Montes S, Rios C (Free copper, ferroxidase and SOD1 activities, lipid peroxidation and NO(x) content in the CSF. A different marker profile in four neurodegenerative diseases. Neurochem Res 33:1717-1723.2008).

Bonifati V, Rizzu P, van Baren MJ, Schaap O, Breedveld GJ, Krieger E, Dekker MC, Squitieri F, Ibanez P, Joosse M, van Dongen JW, Vanacore N, van Swieten JC, Brice A, Meco G, van Duijn CM, Oostra BA, Heutink P (Mutations in the DJ-1 gene associated with autosomal recessive early-onset parkinsonism. Science 299:256-259.2003).

Bonnet AM, Czernecki V (Non-motor symptoms in Parkinson's disease: cognition and behavior. Geriatr Psychol Neuropsychiatr Vieil 11:295-304.2013).

Boulet S, Mounayar S, Poupard A, Bertrand A, Jan C, Pessiglione M, Hirsch EC, Feuerstein C, Francois C, Feger J, Savasta M, Tremblay L (Behavioral recovery in MPTP-treated monkeys: neurochemical mechanisms studied by intrastriatal microdialysis. J Neurosci 28:9575-9584.2008).

Braak H, Del Tredici K, Rub U, de Vos RA, Jansen Steur EN, Braak E (Staging of brain pathology related to sporadic Parkinson's disease. Neurobiol Aging 24:197-211.2003).

Breit S, Schulz JB, Benabid AL (Deep brain stimulation. Cell Tissue Res 318:275-288.2004).

Brooks AI, Chadwick CA, Gelbard HA, Cory-Slechta DA, Federoff HJ (Paraquat elicited neurobehavioral syndrome caused by dopaminergic neuron loss. Brain Res 823:1-10.1999).

Brooks SP, Jones L, Dunnett SB (Comparative analysis of pathology and behavioural phenotypes in mouse models of Huntington's disease. Brain Res Bull 88:81-93.2012).

Brown P, Gerfen CR (Plasticity within striatal direct pathway neurons after neonatal dopamine depletion is mediated through a novel functional coupling of serotonin 5-HT2 receptors to the ERK 1/2 map kinase pathway. J Comp Neurol 498:415-430.2006).

Buhot MC (Serotonin receptors in cognitive behaviors. Curr Opin Neurobiol 7:243-254.1997).

Callio J, Oury TD, Chu CT (Manganese superoxide dismutase protects against 6-hydroxydopamine injury in mouse brains. J Biol Chem 280:18536-18542.2005).

Canet-Aviles RM, Wilson MA, Miller DW, Ahmad R, McLendon C, Bandyopadhyay S, Baptista MJ, Ringe D, Petsko GA, Cookson MR (The Parkinson's disease protein DJ-1 is neuroprotective due to cysteine-sulfinic acid-driven mitochondrial localization. Proc Natl Acad Sci U S A 101:9103-9108.2004).

Cannon JR, Tapias V, Na HM, Honick AS, Drolet RE, Greenamyre JT (A highly reproducible rotenone model of Parkinson's disease. Neurobiol Dis 34:279-290.2009).

Casarejos MJ, Menendez J, Solano RM, Rodriguez-Navarro JA, Garcia de Yebenes J, Mena MA (Susceptibility to rotenone is increased in neurons from parkin null mice and is reduced by minocycline. J Neurochem 97:934-946.2006).

Chandran JS, Lin X, Zapata A, Hoke A, Shimoji M, Moore SO, Galloway MP, Laird FM, Wong PC, Price DL, Bailey KR, Crawley JN, Shippenberg T, Cai H (Progressive behavioral deficits in DJ-1-deficient mice are associated with normal nigrostriatal function. Neurobiol Dis 29:505-514.2008).

Chen L, Cagniard B, Mathews T, Jones S, Koh HC, Ding Y, Carvey PM, Ling Z, Kang UJ, Zhuang X (Age-dependent motor deficits and dopaminergic dysfunction in DJ-1 null mice. J Biol Chem 280:21418-21426.2005).
Chen X, Wu G, Schwarzschild MA (Urate in Parkinson's disease: more than a biomarker? Curr Neurol Neurosci Rep 12:367-375.2012).
Cheng HC, Kim SR, Oo TF, Kareva T, Yarygina O, Rzhetskaya M, Wang C, During M, Talloczy Z, Tanaka K, Komatsu M, Kobayashi K, Okano H, Kholodilov N, Burke RE (Akt suppresses retrograde degeneration of dopaminergic axons by inhibition of macroautophagy. J Neurosci 31:2125-2135.2011).
Chinta SJ, Kumar MJ, Hsu M, Rajagopalan S, Kaur D, Rane A, Nicholls DG, Choi J, Andersen JK (Inducible alterations of glutathione levels in adult dopaminergic midbrain neurons result in nigrostriatal degeneration. J Neurosci 27:13997-14006.2007).
Choi J, Sullards MC, Olzmann JA, Rees HD, Weintraub ST, Bostwick DE, Gearing M, Levey AI, Chin LS, Li L (Oxidative damage of DJ-1 is linked to sporadic Parkinson and Alzheimer diseases. J Biol Chem 281:10816-10824.2006).
Cookson MR (DJ-1, PINK1, and their effects on mitochondrial pathways. Mov Disord 25 Suppl 1:S44-48.2010).
Cooper JA, Sagar HJ, Jordan N, Harvey NS, Sullivan EV (Cognitive impairment in early, untreated Parkinson's disease and its relationship to motor disability. Brain 114 (Pt 5):2095-2122.1991).
Corti O, Lesage S, Brice A (What genetics tells us about the causes and mechanisms of Parkinson's disease. Physiol Rev 91:1161-1218.2011).
Crawley JN (Behavioral phenotyping strategies for mutant mice. Neuron 57:809-818.2008).
Curzon G (The biochemistry of the basal ganglia and Parkinson's disease. Postgrad Med J 53:719-725.1977).
Dawson TM, Ko HS, Dawson VL (Genetic animal models of Parkinson's disease. Neuron 66:646-661.2010).
de Haan JB, Bladier C, Griffiths P, Kelner M, O'Shea RD, Cheung NS, Bronson RT, Silvestro MJ, Wild S, Zheng SS, Beart PM, Hertzog PJ, Kola I (Mice with a homozygous null mutation for the most abundant glutathione peroxidase, Gpx1, show increased susceptibility to the oxidative stress-inducing agents paraquat and hydrogen peroxide. J Biol Chem 273:22528-22536.1998).
de Lau LM, Breteler MM (Epidemiology of Parkinson's disease. Lancet Neurol 5:525-535.2006).
Devos D, Defebvre L, Bordet R (Dopaminergic and non-dopaminergic pharmacological hypotheses for gait disorders in Parkinson's disease. Fundam Clin Pharmacol 24:407-421.2010).
Dexter DT, Carter CJ, Wells FR, Javoy-Agid F, Agid Y, Lees A, Jenner P, Marsden CD (Basal lipid peroxidation in substantia nigra is increased in Parkinson's disease. J Neurochem 52:381-389.1989a).

Dexter DT, Wells FR, Lees AJ, Agid F, Agid Y, Jenner P, Marsden CD (Increased nigral iron content and alterations in other metal ions occurring in brain in Parkinson's disease. J Neurochem 52:1830-1836.1989b).
Di Fonzo A, Rohe CF, Ferreira J, Chien HF, Vacca L, Stocchi F, Guedes L, Fabrizio E, Manfredi M, Vanacore N, Goldwurm S, Breedveld G, Sampaio C, Meco G, Barbosa E, Oostra BA, Bonifati V (A frequent LRRK2 gene mutation associated with autosomal dominant Parkinson's disease. Lancet 365:412-415.2005).
Ding WX, Guo F, Ni HM, Bockus A, Manley S, Stolz DB, Eskelinen EL, Jaeschke H, Yin XM (Parkin and mitofusins reciprocally regulate mitophagy and mitochondrial spheroid formation. J Biol Chem.2012).
Dodson MW, Guo M (Pink1, Parkin, DJ-1 and mitochondrial dysfunction in Parkinson's disease. Curr Opin Neurobiol 17:331-337.2007).
Doty RL, Deems DA, Stellar S (Olfactory dysfunction in parkinsonism: a general deficit unrelated to neurologic signs, disease stage, or disease duration. Neurology 38:1237-1244.1988).
Doty RL, Stern MB, Pfeiffer C, Gollomp SM, Hurtig HI (Bilateral olfactory dysfunction in early stage treated and untreated idiopathic Parkinson's disease. J Neurol Neurosurg Psychiatry 55:138-142.1992).
Duker AP, Espay AJ (Surgical treatment of Parkinson disease: past, present, and future. Neurol Clin 31:799-808.2013).
Dunham NW, Miya TS (A note on a simple apparatus for detecting neurological deficit in rats and mice. J Am Pharm Assoc Am Pharm Assoc (Baltim) 46:208-209.1957).
Eadie MJ (Convulsive ergotism: epidemics of the serotonin syndrome? Lancet Neurol 2:429-434.2003).
Ehringer H, Hornykiewicz O ([Distribution of noradrenaline and dopamine (3-hydroxytyramine) in the human brain and their behavior in diseases of the extrapyramidal system]. Klin Wochenschr 38:1236-1239.1960).
Emre M (Dementia in Parkinson's disease: cause and treatment. Curr Opin Neurol 17:399-404.2004).
Esposito LA, Melov S, Panov A, Cottrell BA, Wallace DC (Mitochondrial disease in mouse results in increased oxidative stress. Proc Natl Acad Sci U S A 96:4820-4825.1999).
Fahn S, Cohen G (The oxidant stress hypothesis in Parkinson's disease: evidence supporting it. Ann Neurol 32:804-812.1992).
Fan J, Ren H, Jia N, Fei E, Zhou T, Jiang P, Wu M, Wang G (DJ-1 decreases Bax expression through repressing p53 transcriptional activity. J Biol Chem 283:4022-4030.2008).
Firestone JA, Lundin JI, Powers KM, Smith-Weller T, Franklin GM, Swanson PD, Longstreth WT, Jr., Checkoway H (Occupational factors and risk of Parkinson's disease: A population-based case-control study. Am J Ind Med 53:217-223.2010).
Fitzmaurice PS, Ang L, Guttman M, Rajput AH, Furukawa Y, Kish SJ (Nigral glutathione deficiency is not specific for idiopathic Parkinson's disease. Mov Disord 18:969-976.2003).

Fleming SM, Chesselet MF (Behavioral phenotypes and pharmacology in genetic mouse models of Parkinsonism. Behav Pharmacol 17:383-391.2006).
Fleming SM, Fernagut PO, Chesselet MF (Genetic mouse models of parkinsonism: strengths and limitations. NeuroRx 2:495-503.2005).
Floor E, Wetzel MG (Increased protein oxidation in human substantia nigra pars compacta in comparison with basal ganglia and prefrontal cortex measured with an improved dinitrophenylhydrazine assay. J Neurochem 70:268-275.1998).
Frank-Cannon TC, Tran T, Ruhn KA, Martinez TN, Hong J, Marvin M, Hartley M, Trevino I, O'Brien DE, Casey B, Goldberg MS, Tansey MG (Parkin deficiency increases vulnerability to inflammation-related nigral degeneration. J Neurosci 28:10825-10834.2008).
Gao H, Yang W, Qi Z, Lu L, Duan C, Zhao C, Yang H (DJ-1 protects dopaminergic neurons against rotenone-induced apoptosis by enhancing ERK-dependent mitophagy. J Mol Biol 423:232-248.2012).
Garcia-Montes JR, Boronat-Garcia A, Lopez-Colome AM, Bargas J, Guerra-Crespo M, Drucker-Colin R (Is nicotine protective against Parkinson's disease? An experimental analysis. CNS Neurol Disord Drug Targets 11:897-906.2012).
Gaspar P, Febvret A, Colombo J (Serotonergic sprouting in primate MTP-induced hemiparkinsonism. Exp Brain Res 96:100-106.1993).
Glasl L, Kloos K, Giesert F, Roethig A, Di Benedetto B, Kuhn R, Zhang J, Hafen U, Zerle J, Hofmann A, Hrabe de Angelis M, Winklhofer KF, Holter SM, Vogt Weisenhorn DM, Wurst W (Pink1-deficiency in mice impairs gait, olfaction and serotonergic innervation of the olfactory bulb. Exp Neurol.2012).
Goetz CG (Charcot on Parkinson's disease. Mov Disord 1:27-32.1986).
Goetz CG (The history of Parkinson's disease: early clinical descriptions and neurological therapies. Cold Spring Harb Perspect Med 1:a008862.2011).
Goldberg MS, Fleming SM, Palacino JJ, Cepeda C, Lam HA, Bhatnagar A, Meloni EG, Wu N, Ackerson LC, Klapstein GJ, Gajendiran M, Roth BL, Chesselet MF, Maidment NT, Levine MS, Shen J (Parkin-deficient mice exhibit nigrostriatal deficits but not loss of dopaminergic neurons. J Biol Chem 278:43628-43635.2003).
Goldberg MS, Pisani A, Haburcak M, Vortherms TA, Kitada T, Costa C, Tong Y, Martella G, Tscherter A, Martins A, Bernardi G, Roth BL, Pothos EN, Calabresi P, Shen J (Nigrostriatal dopaminergic deficits and hypokinesia caused by inactivation of the familial Parkinsonism-linked gene DJ-1. Neuron 45:489-496.2005).
Goldman SM (Environmental Toxins and Parkinson's Disease. Annu Rev Pharmacol Toxicol.2013).
Goldman SM, Quinlan PJ, Ross GW, Marras C, Meng C, Bhudhikanok GS, Comyns K, Korell M, Chade AR, Kasten M, Priestley B, Chou KL, Fernandez HH, Cambi F, Langston JW, Tanner CM (Solvent exposures and Parkinson disease risk in twins. Ann Neurol 71:776-784.2012).

Graham DR, Sidhu A (Mice expressing the A53T mutant form of human alpha-synuclein exhibit hyperactivity and reduced anxiety-like behavior. J Neurosci Res 88:1777-1783.2010).

Guerreiro PS, Huang Y, Gysbers A, Cheng D, Gai WP, Outeiro TF, Halliday GM (LRRK2 interactions with alpha-synuclein in Parkinson's disease brains and in cell models. J Mol Med (Berl) 91:513-522.2013).

Guzman JN, Sanchez-Padilla J, Wokosin D, Kondapalli J, Ilijic E, Schumacker PT, Surmeier DJ (Oxidant stress evoked by pacemaking in dopaminergic neurons is attenuated by DJ-1. Nature 468:696-700.2010).

Halliday GM, McCann H (The progression of pathology in Parkinson's disease. Ann N Y Acad Sci 1184:188-195.2010).

Haque ME, Mount MP, Safarpour F, Abdel-Messih E, Callaghan S, Mazerolle C, Kitada T, Slack RS, Wallace V, Shen J, Anisman H, Park DS (Inactivation of Pink1 gene in vivo sensitizes dopamine-producing neurons to 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) and can be rescued by autosomal recessive Parkinson disease genes, Parkin or DJ-1. J Biol Chem 287:23162-23170.2012).

Hattori N (Autosomal dominant parkinsonism: its etiologies and differential diagnoses. Parkinsonism Relat Disord 18 Suppl 1:S1-3.2012).

Hattori N, Kitada T, Matsumine H, Asakawa S, Yamamura Y, Yoshino H, Kobayashi T, Yokochi M, Wang M, Yoritaka A, Kondo T, Kuzuhara S, Nakamura S, Shimizu N, Mizuno Y (Molecular genetic analysis of a novel Parkin gene in Japanese families with autosomal recessive juvenile parkinsonism: evidence for variable homozygous deletions in the Parkin gene in affected individuals. Ann Neurol 44:935-941.1998).

Hattori N, Sato S (Animal models of Parkinson's disease: similarities and differences between the disease and models. Neuropathology 27:479-483.2007).

Hattoria N, Wanga M, Taka H, Fujimura T, Yoritaka A, Kubo S, Mochizuki H (Toxic effects of dopamine metabolism in Parkinson's disease. Parkinsonism Relat Disord 15 Suppl 1:S35-38.2009).

Hauser DN, Hastings TG (Mitochondrial dysfunction and oxidative stress in Parkinson's disease and monogenic parkinsonism. Neurobiol Dis 51:35-42.2013).

Hayashi T, Ishimori C, Takahashi-Niki K, Taira T, Kim YC, Maita H, Maita C, Ariga H, Iguchi-Ariga SM (DJ-1 binds to mitochondrial complex I and maintains its activity. Biochem Biophys Res Commun 390:667-672.2009).

Hennis MR, Marvin MA, Taylor CM, 2nd, Goldberg MS (Surprising behavioral and neurochemical enhancements in mice with combined mutations linked to Parkinson's disease. Neurobiol Dis.2013).

Hennis MR, Seamans KW, Marvin MA, Casey BH, Goldberg MS (Behavioral and Neurotransmitter Abnormalities in Mice Deficient for Parkin, DJ-1 and Superoxide Dismutase. PLoS One.Submitted).

Hirata H, Asanuma M, Cadet JL (Superoxide radicals are mediators of the effects of methamphetamine on Zif268 (Egr-1, NGFI-A) in the brain: evidence from using CuZn superoxide dismutase transgenic mice. Brain Res Mol Brain Res 58:209-216.1998).

Hirsch E, Graybiel AM, Agid YA (Melanized dopaminergic neurons are differentially susceptible to degeneration in Parkinson's disease. Nature 334:345-348.1988).
Ho YS, Magnenat JL, Bronson RT, Cao J, Gargano M, Sugawara M, Funk CD (Mice deficient in cellular glutathione peroxidase develop normally and show no increased sensitivity to hyperoxia. J Biol Chem 272:16644-16651.1997).
Hoglinger GU, Oertel WH, Hirsch EC (The rotenone model of parkinsonism--the five years inspection. J Neural Transm Suppl 269-272.2006).
Hoglinger GU, Rizk P, Muriel MP, Duyckaerts C, Oertel WH, Caille I, Hirsch EC (Dopamine depletion impairs precursor cell proliferation in Parkinson disease. Nat Neurosci 7:726-735.2004).
Holmes A, Murphy DL, Crawley JN (Reduced aggression in mice lacking the serotonin transporter. Psychopharmacology (Berl) 161:160-167.2002a).
Holmes A, Yang RJ, Murphy DL, Crawley JN (Evaluation of antidepressant-related behavioral responses in mice lacking the serotonin transporter. Neuropsychopharmacology 27:914-923.2002b).
Hornykiewicz O (Dopamine miracle: from brain homogenate to dopamine replacement. Mov Disord 17:501-508.2002a).
Hornykiewicz O (L-DOPA: from a biologically inactive amino acid to a successful therapeutic agent. Amino Acids 23:65-70.2002b).
Hornykiewicz O, Kish SJ (Biochemical pathophysiology of Parkinson's disease. Adv Neurol 45:19-34.1987).
Horowitz MP, Greenamyre JT (Gene-environment interactions in Parkinson's disease: the importance of animal modeling. Clin Pharmacol Ther 88:467-474.2010).
Ihara Y, Chuda M, Kuroda S, Hayabara T (Hydroxyl radical and superoxide dismutase in blood of patients with Parkinson's disease: relationship to clinical data. J Neurol Sci 170:90-95.1999).
Ishikawa S, Taira T, Takahashi-Niki K, Niki T, Ariga H, Iguchi-Ariga SM (Human DJ-1-specific transcriptional activation of tyrosine hydroxylase gene. J Biol Chem 285:39718-39731.2010).
Itier JM, Ibanez P, Mena MA, Abbas N, Cohen-Salmon C, Bohme GA, Laville M, Pratt J, Corti O, Pradier L, Ret G, Joubert C, Periquet M, Araujo F, Negroni J, Casarejos MJ, Canals S, Solano R, Serrano A, Gallego E, Sanchez M, Denefle P, Benavides J, Tremp G, Rooney TA, Brice A, Garcia de Yebenes J (Parkin gene inactivation alters behaviour and dopamine neurotransmission in the mouse. Hum Mol Genet 12:2277-2291.2003).
Jellinger KA (Pathology of Parkinson's disease. Changes other than the nigrostriatal pathway. Mol Chem Neuropathol 14:153-197.1991).
Jiang H, Jiang Q, Feng J (Parkin increases dopamine uptake by enhancing the cell surface expression of dopamine transporter. J Biol Chem 279:54380-54386.2004).
Jiang H, Ren Y, Yuen EY, Zhong P, Ghaedi M, Hu Z, Azabdaftari G, Nakaso K, Yan Z, Feng J (Parkin controls dopamine utilization in human midbrain dopaminergic neurons derived from induced pluripotent stem cells. Nat Commun 3:668.2012).

Joyce PI, Fratta P, Fisher EM, Acevedo-Arozena A (SOD1 and TDP-43 animal models of amyotrophic lateral sclerosis: recent advances in understanding disease toward the development of clinical treatments. Mamm Genome 22:420-448.2011).
Junn E, Jang WH, Zhao X, Jeong BS, Mouradian MM (Mitochondrial localization of DJ-1 leads to enhanced neuroprotection. J Neurosci Res 87:123-129.2009).
Kachroo A, Irizarry MC, Schwarzschild MA (Caffeine protects against combined paraquat and maneb-induced dopaminergic neuron degeneration. Exp Neurol 223:657-661.2010).
Kala SV, Jadhav AL (Low level lead exposure decreases in vivo release of dopamine in the rat nucleus accumbens: a microdialysis study. J Neurochem 65:1631-1635.1995).
Kaur D, Andersen J (Does cellular iron dysregulation play a causative role in Parkinson's disease? Ageing Res Rev 3:327-343.2004).
Kawajiri S, Saiki S, Sato S, Sato F, Hatano T, Eguchi H, Hattori N (PINK1 is recruited to mitochondria with parkin and associates with LC3 in mitophagy. FEBS Lett 584:1073-1079.2010).
Keithley EM, Canto C, Zheng QY, Wang X, Fischel-Ghodsian N, Johnson KR (Cu/Zn superoxide dismutase and age-related hearing loss. Hear Res 209:76-85.2005).
Kempster PA, Hurwitz B, Lees AJ (A new look at James Parkinson's Essay on the Shaking Palsy. Neurology 69:482-485.2007).
Khan W, Priyadarshini M, Zakai HA, Kamal MA, Alam Q (A brief overview of tyrosine hydroxylase and alpha-synuclein in the Parkinsonian brain. CNS Neurol Disord Drug Targets 11:456-462.2012).
Kieburtz K, Wunderle KB (Parkinson's disease: evidence for environmental risk factors. Mov Disord 28:8-13.2013).
Kim RH, Smith PD, Aleyasin H, Hayley S, Mount MP, Pownall S, Wakeham A, You-Ten AJ, Kalia SK, Horne P, Westaway D, Lozano AM, Anisman H, Park DS, Mak TW (Hypersensitivity of DJ-1-deficient mice to 1-methyl-4-phenyl-1,2,3,6-tetrahydropyrindine (MPTP) and oxidative stress. Proc Natl Acad Sci U S A 102:5215-5220.2005).
Kish SJ, Morito C, Hornykiewicz O (Glutathione peroxidase activity in Parkinson's disease brain. Neurosci Lett 58:343-346.1985).
Kish SJ, Tong J, Hornykiewicz O, Rajput A, Chang LJ, Guttman M, Furukawa Y (Preferential loss of serotonin markers in caudate versus putamen in Parkinson's disease. Brain 131:120-131.2008).
Kitada T, Asakawa S, Hattori N, Matsumine H, Yamamura Y, Minoshima S, Yokochi M, Mizuno Y, Shimizu N (Mutations in the parkin gene cause autosomal recessive juvenile parkinsonism. Nature 392:605-608.1998).
Kitada T, Tong Y, Gautier CA, Shen J (Absence of nigral degeneration in aged parkin/DJ-1/PINK1 triple knockout mice. J Neurochem 111:696-702.2009).
Klivenyi P, Andreassen OA, Ferrante RJ, Dedeoglu A, Mueller G, Lancelot E, Bogdanov M, Andersen JK, Jiang D, Beal MF (Mice deficient in cellular glutathione peroxidase show increased vulnerability to malonate, 3-nitropropionic acid, and 1-methyl-4-phenyl-1,2,5,6-tetrahydropyridine. J Neurosci 20:1-7.2000).

Kostrzewa RM, Reader TA, Descarries L (Serotonin neural adaptations to ontogenetic loss of dopamine neurons in rat brain. J Neurochem 70:889-898.1998).
Kubo SI, Kitami T, Noda S, Shimura H, Uchiyama Y, Asakawa S, Minoshima S, Shimizu N, Mizuno Y, Hattori N (Parkin is associated with cellular vesicles. J Neurochem 78:42-54.2001).
Kuhn K, Zhu XR, Lubbert H, Stichel CC (Parkin expression in the developing mouse. Brain Res Dev Brain Res 149:131-142.2004).
Kunikowska G, Jenner P (Alterations in m-RNA expression for Cu,Zn-superoxide dismutase and glutathione peroxidase in the basal ganglia of MPTP-treated marmosets and patients with Parkinson's disease. Brain Res 968:206-218.2003).
Kuroda Y, Mitsui T, Kunishige M, Shono M, Akaike M, Azuma H, Matsumoto T (Parkin enhances mitochondrial biogenesis in proliferating cells. Hum Mol Genet 15:883-895.2006).
Langston JW, Ballard P, Tetrud JW, Irwin I (Chronic Parkinsonism in humans due to a product of meperidine-analog synthesis. Science 219:979-980.1983).
Lee CS, Sauer H, Bjorklund A (Dopaminergic neuronal degeneration and motor impairments following axon terminal lesion by instrastriatal 6-hydroxydopamine in the rat. Neuroscience 72:641-653.1996).
Lee LJ (Neonatal fluoxetine exposure alters motor performances of adolescent rats. Dev Neurobiol 72:1122-1132.2012).
Lees AJ, Selikhova M, Andrade LA, Duyckaerts C (The black stuff and Konstantin Nikolaevich Tretiakoff. Mov Disord 23:777-783.2008).
Lees AJ, Smith E (Cognitive deficits in the early stages of Parkinson's disease. Brain 106 (Pt 2):257-270.1983).
Lenzken SC, Romeo V, Zolezzi F, Cordero F, Lamorte G, Bonanno D, Biancolini D, Cozzolino M, Pesaresi MG, Maracchioni A, Sanges R, Achsel T, Carri MT, Calogero RA, Barabino SM (Mutant SOD1 and mitochondrial damage alter expression and splicing of genes controlling neuritogenesis in models of neurodegeneration. Hum Mutat 32:168-182.2011).
Lesemann A, Reinel C, Huhnchen P, Pilhatsch M, Hellweg R, Klaissle P, Winter C, Steiner B (MPTP-induced hippocampal effects on serotonin, dopamine, neurotrophins, adult neurogenesis and depression-like behavior are partially influenced by fluoxetine in adult mice. Brain Res.2012).
Lev N, Barhum Y, Ben-Zur T, Melamed E, Steiner I, Offen D (Knocking out DJ-1 attenuates astrocytes neuroprotection against 6-hydroxydopamine toxicity. J Mol Neurosci 50:542-550.2013).
Lev N, Ickowicz D, Melamed E, Offen D (Oxidative insults induce DJ-1 upregulation and redistribution: implications for neuroprotection. Neurotoxicology 29:397-405.2008).
Levin BE, Llabre MM, Weiner WJ (Cognitive impairments associated with early Parkinson's disease. Neurology 39:557-561.1989).
Levy G, Jacobs DM, Tang MX, Cote LJ, Louis ED, Alfaro B, Mejia H, Stern Y, Marder K (Memory and executive function impairment predict dementia in Parkinson's disease. Mov Disord 17:1221-1226.2002a).

Levy G, Schupf N, Tang MX, Cote LJ, Louis ED, Mejia H, Stern Y, Marder K (Combined effect of age and severity on the risk of dementia in Parkinson's disease. Ann Neurol 51:722-729.2002b).

Li L, Qiu G, Ding S, Zhou FM (Serotonin hyperinnervation and upregulated 5-HT2A receptor expression and motor-stimulating function in nigrostriatal dopamine-deficient Pitx3 mutant mice. Brain Res 1491:236-250.2013).

Li Y, Huang TT, Carlson EJ, Melov S, Ursell PC, Olson JL, Noble LJ, Yoshimura MP, Berger C, Chan PH, Wallace DC, Epstein CJ (Dilated cardiomyopathy and neonatal lethality in mutant mice lacking manganese superoxide dismutase. Nat Genet 11:376-381.1995).

Liang LP, Patel M (Iron-sulfur enzyme mediated mitochondrial superoxide toxicity in experimental Parkinson's disease. J Neurochem 90:1076-1084.2004).

Liddell JR, Robinson SR, Dringen R, Bishop GM (Astrocytes retain their antioxidant capacity into advanced old age. Glia 58:1500-1509.2010).

Lindgren HS, Dunnett SB (Cognitive dysfunction and depression in Parkinson's disease: what can be learned from rodent models? Eur J Neurosci 35:1894-1907.2012).

Lo Bianco C, Schneider BL, Bauer M, Sajadi A, Brice A, Iwatsubo T, Aebischer P (Lentiviral vector delivery of parkin prevents dopaminergic degeneration in an alpha-synuclein rat model of Parkinson's disease. Proc Natl Acad Sci U S A 101:17510-17515.2004).

Lopez G, Sidransky E (Autosomal recessive mutations in the development of Parkinson's disease. Biomark Med 4:713-721.2010).

Louis ED (The shaking palsy, the first forty-five years: a journey through the British literature. Mov Disord 12:1068-1072.1997).

Maeda T, Kannari K, Shen H, Arai A, Tomiyama M, Matsunaga M, Suda T (Rapid induction of serotonergic hyperinnervation in the adult rat striatum with extensive dopaminergic denervation. Neurosci Lett 343:17-20.2003).

Maeda T, Nagata K, Yoshida Y, Kannari K (Serotonergic hyperinnervation into the dopaminergic denervated striatum compensates for dopamine conversion from exogenously administered l-DOPA. Brain Res 1046:230-233.2005).

Mahieux F, Fenelon G, Flahault A, Manifacier MJ, Michelet D, Boller F (Neuropsychological prediction of dementia in Parkinson's disease. J Neurol Neurosurg Psychiatry 64:178-183.1998).

Mak SK, McCormack AL, Langston JW, Kordower JH, Di Monte DA (Decreased alpha-synuclein expression in the aging mouse substantia nigra. Exp Neurol 220:359-365.2009).

Mandillo S, Golini E, Marazziti D, Di Pietro C, Matteoni R, Tocchini-Valentini GP (Mice lacking the Parkinson's related GPR37/PAEL receptor show non-motor behavioral phenotypes: age and gender effect. Genes Brain Behav 12:465-477.2013).

Manning-Bog AB, Caudle WM, Perez XA, Reaney SH, Paletzki R, Isla MZ, Chou VP, McCormack AL, Miller GW, Langston JW, Gerfen CR, Dimonte DA (Increased vulnerability of nigrostriatal terminals in DJ-1-deficient mice is mediated by the dopamine transporter. Neurobiol Dis 27:141-150.2007).

Mathur BN, Lovinger DM (Serotonergic action on dorsal striatal function. Parkinsonism Relat Disord 18 Suppl 1:S129-131.2012).
McCormack AL, Atienza JG, Johnston LC, Andersen JK, Vu S, Di Monte DA (Role of oxidative stress in paraquat-induced dopaminergic cell degeneration. J Neurochem 93:1030-1037.2005).
McFadden SL, Ding D, Burkard RF, Jiang H, Reaume AG, Flood DG, Salvi RJ (Cu/Zn SOD deficiency potentiates hearing loss and cochlear pathology in aged 129,CD-1 mice. J Comp Neurol 413:101-112.1999).
Menzies FM, Yenisetti SC, Min KT (Roles of Drosophila DJ-1 in survival of dopaminergic neurons and oxidative stress. Curr Biol 15:1578-1582.2005).
Meulener M, Whitworth AJ, Armstrong-Gold CE, Rizzu P, Heutink P, Wes PD, Pallanck LJ, Bonini NM (Drosophila DJ-1 mutants are selectively sensitive to environmental toxins associated with Parkinson's disease. Curr Biol 15:1572-1577.2005).
Miyama A, Saito Y, Yamanaka K, Hayashi K, Hamakubo T, Noguchi N (Oxidation of DJ-1 induced by 6-hydroxydopamine decreasing intracellular glutathione. PLoS One 6:e27883.2011).
Morelli E, Moore H, Rebello TJ, Gray N, Steele K, Esposito E, Gingrich JA, Ansorge MS (Chronic 5-HT transporter blockade reduces DA signaling to elicit basal ganglia dysfunction. J Neurosci 31:15742-15750.2011).
Mortiboys H, Thomas KJ, Koopman WJ, Klaffke S, Abou-Sleiman P, Olpin S, Wood NW, Willems PH, Smeitink JA, Cookson MR, Bandmann O (Mitochondrial function and morphology are impaired in parkin-mutant fibroblasts. Ann Neurol 64:555-565.2008).
Muftuoglu M, Elibol B, Dalmizrak O, Ercan A, Kulaksiz G, Ogus H, Dalkara T, Ozer N (Mitochondrial complex I and IV activities in leukocytes from patients with parkin mutations. Mov Disord 19:544-548.2004).
Mullett SJ, Hinkle DA (DJ-1 knock-down in astrocytes impairs astrocyte-mediated neuroprotection against rotenone. Neurobiol Dis 33:28-36.2009).
Mullett SJ, Hinkle DA (DJ-1 deficiency in astrocytes selectively enhances mitochondrial Complex I inhibitor-induced neurotoxicity. J Neurochem 117:375-387.2011).
Munoz-Soriano V, Nieto-Arellano R, Paricio N (Septin 4, the drosophila ortholog of human CDCrel-1, accumulates in parkin mutant brains and is functionally related to the Nedd4 E3 ubiquitin ligase. J Mol Neurosci 48:136-143.2012).
Narendra D, Tanaka A, Suen DF, Youle RJ (Parkin is recruited selectively to impaired mitochondria and promotes their autophagy. J Cell Biol 183:795-803.2008).
Nichols WC, Pankratz N, Hernandez D, Paisan-Ruiz C, Jain S, Halter CA, Michaels VE, Reed T, Rudolph A, Shults CW, Singleton A, Foroud T (Genetic screening for a single common LRRK2 mutation in familial Parkinson's disease. Lancet 365:410-412.2005).
Nishinaga H, Takahashi-Niki K, Taira T, Andreadis A, Iguchi-Ariga SM, Ariga H (Expression profiles of genes in DJ-1-knockdown and L 166 P DJ-1 mutant cells. Neurosci Lett 390:54-59.2005).

Norrholm SD, Horton DB, Dwoskin LP (The promiscuity of the dopamine transporter: implications for the kinetic analysis of [3H]serotonin uptake in rat hippocampal and striatal synaptosomes. Neuropharmacology 53:982-989.2007).
Nussbaum RL, Polymeropoulos MH (Genetics of Parkinson's disease. Hum Mol Genet 6:1687-1691.1997).
Ohlemiller KK, McFadden SL, Ding DL, Lear PM, Ho YS (Targeted mutation of the gene for cellular glutathione peroxidase (Gpx1) increases noise-induced hearing loss in mice. J Assoc Res Otolaryngol 1:243-254.2000).
Okatsu K, Iemura S, Koyano F, Go E, Kimura M, Natsume T, Tanaka K, Matsuda N (Mitochondrial hexokinase HKI is a novel substrate of the Parkin ubiquitin ligase. Biochem Biophys Res Commun 428:197-202.2012).
Olzmann JA, Brown K, Wilkinson KD, Rees HD, Huai Q, Ke H, Levey AI, Li L, Chin LS (Familial Parkinson's disease-associated L166P mutation disrupts DJ-1 protein folding and function. J Biol Chem 279:8506-8515.2004).
Omura T, Kaneko M, Okuma Y, Orba Y, Nagashima K, Takahashi R, Fujitani N, Matsumura S, Hata A, Kubota K, Murahashi K, Uehara T, Nomura Y (A ubiquitin ligase HRD1 promotes the degradation of Pael receptor, a substrate of Parkin. J Neurochem 99:1456-1469.2006).
Oyama G, Yoshimi K, Natori S, Chikaoka Y, Ren YR, Funayama M, Shimo Y, Takahashi R, Nakazato T, Kitazawa S, Hattori N (Impaired in vivo dopamine release in parkin knockout mice. Brain Res 1352:214-222.2010).
Paisan-Ruiz C, Jain S, Evans EW, Gilks WP, Simon J, van der Brug M, Lopez de Munain A, Aparicio S, Gil AM, Khan N, Johnson J, Martinez JR, Nicholl D, Carrera IM, Pena AS, de Silva R, Lees A, Marti-Masso JF, Perez-Tur J, Wood NW, Singleton AB (Cloning of the gene containing mutations that cause PARK8-linked Parkinson's disease. Neuron 44:595-600.2004).
Palacino JJ, Sagi D, Goldberg MS, Krauss S, Motz C, Wacker M, Klose J, Shen J (Mitochondrial dysfunction and oxidative damage in parkin-deficient mice. J Biol Chem 279:18614-18622.2004).
Parent M, Parent A (Substantia nigra and Parkinson's disease: a brief history of their long and intimate relationship. Can J Neurol Sci 37:313-319.2010).
Park HR, Kim J, Kim T, Jo S, Yeom M, Moon B, Choo IH, Lee J, Lim EJ, Park KD, Min SJ, Nam G, Keum G, Lee CJ, Choo H (Oxazolopyridines and thiazolopyridines as monoamine oxidase B inhibitors for the treatment of Parkinson's disease. Bioorg Med Chem 21:5480-5487.2013).
Parkinson J (An essay on the shaking palsy. 1817. J Neuropsychiatry Clin Neurosci 14:223-236; discussion 222.2002).
Paterna JC, Leng A, Weber E, Feldon J, Bueler H (DJ-1 and Parkin modulate dopamine-dependent behavior and inhibit MPTP-induced nigral dopamine neuron loss in mice. Mol Ther 15:698-704.2007).
Paus M, Kohl Z, Ben Abdallah NM, Galter D, Gillardon F, Winkler J (Enhanced dendritogenesis and axogenesis in hippocampal neuroblasts of LRRK2 knockout mice. Brain Res 1497:85-100.2013).

Pavao R, Helene AF, Xavier GF (Parkinson's disease progression: implicit acquisition, cognitive and motor impairments, and medication effects. Front Integr Neurosci 6:56.2012).
Pellicano C, Benincasa D, Pisani V, Buttarelli FR, Giovannelli M, Pontieri FE (Prodromal non-motor symptoms of Parkinson's disease. Neuropsychiatr Dis Treat 3:145-152.2007).
Perez FA, Curtis WR, Palmiter RD (Parkin-deficient mice are not more sensitive to 6-hydroxydopamine or methamphetamine neurotoxicity. BMC Neurosci 6:71.2005).
Perez FA, Palmiter RD (Parkin-deficient mice are not a robust model of parkinsonism. Proc Natl Acad Sci U S A 102:2174-2179.2005).
Perucho J, Casarejos MJ, Rubio I, Rodriguez-Navarro JA, Gomez A, Ampuero I, Rodal I, Solano RM, Carro E, Garcia de Yebenes J, Mena MA (The effects of parkin suppression on the behaviour, amyloid processing, and cell survival in APP mutant transgenic mice. Exp Neurol 221:54-67.2010).
Pham AH, Meng S, Chu QN, Chan DC (Loss of Mfn2 results in progressive, retrograde degeneration of dopaminergic neurons in the nigrostriatal circuit. Hum Mol Genet 21:4817-4826.2012).
Pham TT, Giesert F, Rothig A, Floss T, Kallnik M, Weindl K, Holter SM, Ahting U, Prokisch H, Becker L, Klopstock T, Hrabe de Angelis M, Beyer K, Gorner K, Kahle PJ, Vogt Weisenhorn DM, Wurst W (DJ-1-deficient mice show less TH-positive neurons in the ventral tegmental area and exhibit non-motoric behavioural impairments. Genes Brain Behav 9:305-317.2010).
Pisani A, Martella G, Tscherter A, Costa C, Mercuri NB, Bernardi G, Shen J, Calabresi P (Enhanced sensitivity of DJ-1-deficient dopaminergic neurons to energy metabolism impairment: role of Na+/K+ ATPase. Neurobiol Dis 23:54-60.2006).
Politis M, Wu K, Loane C, Kiferle L, Molloy S, Brooks DJ, Piccini P (Staging of serotonergic dysfunction in Parkinson's disease: an in vivo 11C-DASB PET study. Neurobiol Dis 40:216-221.2010a).
Politis M, Wu K, Loane C, Quinn NP, Brooks DJ, Rehncrona S, Bjorklund A, Lindvall O, Piccini P (Serotonergic neurons mediate dyskinesia side effects in Parkinson's patients with neural transplants. Sci Transl Med 2:38ra46.2010b).
Polymeropoulos MH, Lavedan C, Leroy E, Ide SE, Dehejia A, Dutra A, Pike B, Root H, Rubenstein J, Boyer R, Stenroos ES, Chandrasekharappa S, Athanassiadou A, Papapetropoulos T, Johnson WG, Lazzarini AM, Duvoisin RC, Di Iorio G, Golbe LI, Nussbaum RL (Mutation in the alpha-synuclein gene identified in families with Parkinson's disease. Science 276:2045-2047.1997).
Puschmann A (Monogenic Parkinson's disease and parkinsonism: clinical phenotypes and frequencies of known mutations. Parkinsonism Relat Disord 19:407-415.2013).
Raisman R, Cash R, Agid Y (Parkinson's disease: decreased density of 3H-imipramine and 3H-paroxetine binding sites in putamen. Neurology 36:556-560.1986).
Ramaswamy S, McBride JL, Han I, Berry-Kravis EM, Zhou L, Herzog CD, Gasmi M, Bartus RT, Kordower JH (Intrastriatal CERE-120 (AAV-Neurturin) protects

striatal and cortical neurons and delays motor deficits in a transgenic mouse model of Huntington's disease. Neurobiol Dis 34:40-50.2009).

Rampersaud N, Harkavyi A, Giordano G, Lever R, Whitton J, Whitton PS (Exendin-4 reverses biochemical and behavioral deficits in a pre-motor rodent model of Parkinson's disease with combined noradrenergic and serotonergic lesions. Neuropeptides 46:183-193.2012).

Ren Y, Feng J (Rotenone selectively kills serotonergic neurons through a microtubule-dependent mechanism. J Neurochem 103:303-311.2007).

Riederer P, Laux G (MAO-inhibitors in Parkinson's Disease. Exp Neurobiol 20:1-17.2011).

Rodriguez-Navarro JA, Casarejos MJ, Menendez J, Solano RM, Rodal I, Gomez A, Yebenes JG, Mena MA (Mortality, oxidative stress and tau accumulation during ageing in parkin null mice. J Neurochem 103:98-114.2007).

Roiser JP, Blackwell AD, Cools R, Clark L, Rubinsztein DC, Robbins TW, Sahakian BJ (Serotonin transporter polymorphism mediates vulnerability to loss of incentive motivation following acute tryptophan depletion. Neuropsychopharmacology 31:2264-2272.2006).

Roselli F, Pisciotta NM, Pennelli M, Aniello MS, Gigante A, De Caro MF, Ferrannini E, Tartaglione B, Niccoli-Asabella A, Defazio G, Livrea P, Rubini G (Midbrain SERT in degenerative parkinsonisms: a 123I-FP-CIT SPECT study. Mov Disord 25:1853-1859.2010).

Rousseaux MW, Marcogliese PC, Qu D, Hewitt SJ, Seang S, Kim RH, Slack RS, Schlossmacher MG, Lagace DC, Mak TW, Park DS (Progressive dopaminergic cell loss with unilateral-to-bilateral progression in a genetic model of Parkinson disease. Proc Natl Acad Sci U S A 109:15918-15923.2012).

Rozas G, Liste I, Guerra MJ, Labandeira-Garcia JL (Sprouting of the serotonergic afferents into striatum after selective lesion of the dopaminergic system by MPTP in adult mice. Neurosci Lett 245:151-154.1998).

Sato S, Chiba T, Nishiyama S, Kakiuchi T, Tsukada H, Hatano T, Fukuda T, Yasoshima Y, Kai N, Kobayashi K, Mizuno Y, Tanaka K, Hattori N (Decline of striatal dopamine release in parkin-deficient mice shown by ex vivo autoradiography. J Neurosci Res 84:1350-1357.2006).

Scatton B, Javoy-Agid F, Rouquier L, Dubois B, Agid Y (Reduction of cortical dopamine, noradrenaline, serotonin and their metabolites in Parkinson's disease. Brain Res 275:321-328.1983).

Schober A (Classic toxin-induced animal models of Parkinson's disease: 6-OHDA and MPTP. Cell Tissue Res 318:215-224.2004).

Seet RC, Lee CY, Lim EC, Tan JJ, Quek AM, Chong WL, Looi WF, Huang SH, Wang H, Chan YH, Halliwell B (Oxidative damage in Parkinson disease: Measurement using accurate biomarkers. Free Radic Biol Med 48:560-566.2010).

Segal NL (Comment on "solvent exposures and Parkinson disease risk in twins". Ann Neurol 72:294; author reply 294-295.2012).

Shang H, Lang D, Jean-Marc B, Kaelin-Lang A (Localization of DJ-1 mRNA in the mouse brain. Neurosci Lett 367:273-277.2004).

Shimura H, Hattori N, Kubo S, Mizuno Y, Asakawa S, Minoshima S, Shimizu N, Iwai K, Chiba T, Tanaka K, Suzuki T (Familial Parkinson disease gene product, parkin, is a ubiquitin-protein ligase. Nat Genet 25:302-305.2000).
Shimura H, Hattori N, Kubo S, Yoshikawa M, Kitada T, Matsumine H, Asakawa S, Minoshima S, Yamamura Y, Shimizu N, Mizuno Y (Immunohistochemical and subcellular localization of Parkin protein: absence of protein in autosomal recessive juvenile parkinsonism patients. Ann Neurol 45:668-672.1999).
Shulman JM, De Jager PL, Feany MB (Parkinson's disease: genetics and pathogenesis. Annu Rev Pathol 6:193-222.2011).
Sian J, Dexter DT, Lees AJ, Daniel S, Agid Y, Javoy-Agid F, Jenner P, Marsden CD (Alterations in glutathione levels in Parkinson's disease and other neurodegenerative disorders affecting basal ganglia. Ann Neurol 36:348-355.1994).
Spencer JP, Jenner A, Aruoma OI, Evans PJ, Kaur H, Dexter DT, Jenner P, Lees AJ, Marsden DC, Halliwell B (Intense oxidative DNA damage promoted by L-dopa and its metabolites. Implications for neurodegenerative disease. FEBS Lett 353:246-250.1994).
Stichel CC, Augustin M, Kuhn K, Zhu XR, Engels P, Ullmer C, Lubbert H (Parkin expression in the adult mouse brain. Eur J Neurosci 12:4181-4194.2000).
Sun SY, An CN, Pu XP (DJ-1 protein protects dopaminergic neurons against 6-OHDA/MG-132-induced neurotoxicity in rats. Brain Res Bull 88:609-616.2012).
Szot P, Franklin A, Sikkema C, Wilkinson CW, Raskind MA (Sequential Loss of LC Noradrenergic and Dopaminergic Neurons Results in a Correlation of Dopaminergic Neuronal Number to Striatal Dopamine Concentration. Front Pharmacol 3:184.2012).
Taira T, Saito Y, Niki T, Iguchi-Ariga SM, Takahashi K, Ariga H (DJ-1 has a role in antioxidative stress to prevent cell death. EMBO Rep 5:213-218.2004).
Tanner CM, Kamel F, Ross GW, Hoppin JA, Goldman SM, Korell M, Marras C, Bhudhikanok GS, Kasten M, Chade AR, Comyns K, Richards MB, Meng C, Priestley B, Fernandez HH, Cambi F, Umbach DM, Blair A, Sandler DP, Langston JW (Rotenone, paraquat, and Parkinson's disease. Environ Health Perspect 119:866-872.2011).
Tanner CM, Ross GW, Jewell SA, Hauser RA, Jankovic J, Factor SA, Bressman S, Deligtisch A, Marras C, Lyons KE, Bhudhikanok GS, Roucoux DF, Meng C, Abbott RD, Langston JW (Occupation and risk of parkinsonism: a multicenter case-control study. Arch Neurol 66:1106-1113.2009).
Taylor TN, Caudle WM, Shepherd KR, Noorian A, Jackson CR, Iuvone PM, Weinshenker D, Greene JG, Miller GW (Nonmotor symptoms of Parkinson's disease revealed in an animal model with reduced monoamine storage capacity. J Neurosci 29:8103-8113.2009).
Thomas KJ, McCoy MK, Blackinton J, Beilina A, van der Brug M, Sandebring A, Miller D, Maric D, Cedazo-Minguez A, Cookson MR (DJ-1 acts in parallel to the PINK1/parkin pathway to control mitochondrial function and autophagy. Hum Mol Genet 20:40-50.2011).

Trempe JF, Fon EA (Structure and Function of Parkin, PINK1, and DJ-1, the Three Musketeers of Neuroprotection. Front Neurol 4:38.2013).

Tripanichkul W, Sripanichkulchai K, Duce JA, Finkelstein DI (17Beta-estradiol reduces nitrotyrosine immunoreactivity and increases SOD1 and SOD2 immunoreactivity in nigral neurons in male mice following MPTP insult. Brain Res 1164:24-31.2007).

Uitti RJ, Snow BJ, Shinotoh H, Vingerhoets FJ, Hayward M, Hashimoto S, Richmond J, Markey SP, Markey CJ, Calne DB (Parkinsonism induced by solvent abuse. Ann Neurol 35:616-619.1994).

Ulusoy A, Kirik D (Can overexpression of parkin provide a novel strategy for neuroprotection in Parkinson's disease? Exp Neurol 212:258-260.2008).

Um JW, Im E, Lee HJ, Min B, Yoo L, Yoo J, Lubbert H, Stichel-Gunkel C, Cho HS, Yoon JB, Chung KC (Parkin directly modulates 26S proteasome activity. J Neurosci 30:11805-11814.2010).

Usami Y, Hatano T, Imai S, Kubo S, Sato S, Saiki S, Fujioka Y, Ohba Y, Sato F, Funayama M, Eguchi H, Shiba K, Ariga H, Shen J, Hattori N (DJ-1 associates with synaptic membranes. Neurobiol Dis 43:651-662.2011).

Valente EM, Bentivoglio AR, Dixon PH, Ferraris A, Ialongo T, Frontali M, Albanese A, Wood NW (Localization of a novel locus for autosomal recessive early-onset parkinsonism, PARK6, on human chromosome 1p35-p36. Am J Hum Genet 68:895-900.2001).

Valente EM, Brancati F, Ferraris A, Graham EA, Davis MB, Breteler MM, Gasser T, Bonifati V, Bentivoglio AR, De Michele G, Durr A, Cortelli P, Wassilowsky D, Harhangi BS, Rawal N, Caputo V, Filla A, Meco G, Oostra BA, Brice A, Albanese A, Dallapiccola B, Wood NW (PARK6-linked parkinsonism occurs in several European families. Ann Neurol 51:14-18.2002).

Van Remmen H, Salvador C, Yang H, Huang TT, Epstein CJ, Richardson A (Characterization of the antioxidant status of the heterozygous manganese superoxide dismutase knockout mouse. Arch Biochem Biophys 363:91-97.1999).

Varcin M, Bentea E, Michotte Y, Sarre S (Oxidative stress in genetic mouse models of Parkinson's disease. Oxid Med Cell Longev 2012:624925.2012).

Venkateshappa C, Harish G, Mythri RB, Mahadevan A, Bharath MM, Shankar SK (Increased oxidative damage and decreased antioxidant function in aging human substantia nigra compared to striatum: implications for Parkinson's disease. Neurochem Res 37:358-369.2012).

Vercammen L, Van der Perren A, Vaudano E, Gijsbers R, Debyser Z, Van den Haute C, Baekelandt V (Parkin protects against neurotoxicity in the 6-hydroxydopamine rat model for Parkinson's disease. Mol Ther 14:716-723.2006).

Vernon AC (Mice with reduced vesicular monoamine storage content display nonmotor features of Parkinson's disease. J Neurosci 29:12842-12844.2009).

Vincelette J, Xu Y, Zhang LN, Schaefer CJ, Vergona R, Sullivan ME, Hampton TG, Wang YX (Gait analysis in a murine model of collagen-induced arthritis. Arthritis Res Ther 9:R123.2007).

Vincent A, Briggs L, Chatwin GF, Emery E, Tomlins R, Oswald M, Middleton CA, Evans GJ, Sweeney ST, Elliott CJ (parkin-induced defects in neurophysiology and locomotion are generated by metabolic dysfunction and not oxidative stress. Hum Mol Genet 21:1760-1769.2012).
Vincow ES, Merrihew G, Thomas RE, Shulman NJ, Beyer RP, Maccoss MJ, Pallanck LJ (The PINK1-Parkin pathway promotes both mitophagy and selective respiratory chain turnover in vivo. Proc Natl Acad Sci U S A 110:6400-6405.2013).
Vinish M, Anand A, Prabhakar S (Altered oxidative stress levels in Indian Parkinson's disease patients with PARK2 mutations. Acta Biochim Pol 58:165-169.2011).
Von Coelln R, Thomas B, Savitt JM, Lim KL, Sasaki M, Hess EJ, Dawson VL, Dawson TM (Loss of locus coeruleus neurons and reduced startle in parkin null mice. Proc Natl Acad Sci U S A 101:10744-10749.2004).
Wakabayashi K (Cellular pathology of neurodegenerative disorders. Rinsho Shinkeigaku 53:609-617.2013).
Wang D, Qian L, Xiong H, Liu J, Neckameyer WS, Oldham S, Xia K, Wang J, Bodmer R, Zhang Z (Antioxidants protect PINK1-dependent dopaminergic neurons in Drosophila. Proc Natl Acad Sci U S A 103:13520-13525.2006).
Wang HQ, Imai Y, Inoue H, Kataoka A, Iita S, Nukina N, Takahashi R (Pael-R transgenic mice crossed with parkin deficient mice displayed progressive and selective catecholaminergic neuronal loss. J Neurochem 107:171-185.2008).
Wang X, Winter D, Ashrafi G, Schlehe J, Wong YL, Selkoe D, Rice S, Steen J, LaVoie MJ, Schwarz TL (PINK1 and Parkin target Miro for phosphorylation and degradation to arrest mitochondrial motility. Cell 147:893-906.2011a).
Wang Z, Liu J, Chen S, Wang Y, Cao L, Zhang Y, Kang W, Li H, Gui Y, Ding J (DJ-1 modulates the expression of Cu/Zn-superoxide dismutase-1 through the Erk1/2-Elk1 pathway in neuroprotection. Ann Neurol 70:591-599.2011b).
Warner TT, Schapira AH (Genetic and environmental factors in the cause of Parkinson's disease. Ann Neurol 53 Suppl 3:S16-23; discussion S23-15.2003).
Weaver FM, Follett K, Stern M, Hur K, Harris C, Marks WJ, Jr., Rothlind J, Sagher O, Reda D, Moy CS, Pahwa R, Burchiel K, Hogarth P, Lai EC, Duda JE, Holloway K, Samii A, Horn S, Bronstein J, Stoner G, Heemskerk J, Huang GD (Bilateral deep brain stimulation vs best medical therapy for patients with advanced Parkinson disease: a randomized controlled trial. JAMA 301:63-73.2009).
Weisskopf MG, Weuve J, Nie H, Saint-Hilaire MH, Sudarsky L, Simon DK, Hersh B, Schwartz J, Wright RO, Hu H (Association of cumulative lead exposure with Parkinson's disease. Environ Health Perspect 118:1609-1613.2010).
Whitworth AJ, Theodore DA, Greene JC, Benes H, Wes PD, Pallanck LJ (Increased glutathione S-transferase activity rescues dopaminergic neuron loss in a Drosophila model of Parkinson's disease. Proc Natl Acad Sci U S A 102:8024-8029.2005).
Williams MD, Van Remmen H, Conrad CC, Huang TT, Epstein CJ, Richardson A (Increased oxidative damage is correlated to altered mitochondrial function in heterozygous manganese superoxide dismutase knockout mice. J Biol Chem 273:28510-28515.1998).

Xie Z, Zhuang X, Chen L (DJ-1 mRNA anatomical localization and cell type identification in the mouse brain. Neurosci Lett 465:214-219.2009).
Xu W, Barrientos T, Andrews NC (Iron and copper in mitochondrial diseases. Cell Metab 17:319-328.2013).
Yamakado H, Moriwaki Y, Yamasaki N, Miyakawa T, Kurisu J, Uemura K, Inoue H, Takahashi M, Takahashi R (alpha-Synuclein BAC transgenic mice as a model for Parkinson's disease manifested decreased anxiety-like behavior and hyperlocomotion. Neurosci Res 73:173-177.2012).
Yang W, Chen L, Ding Y, Zhuang X, Kang UJ (Paraquat induces dopaminergic dysfunction and proteasome impairment in DJ-1-deficient mice. Hum Mol Genet 16:2900-2910.2007).
Yang Y, Gehrke S, Haque ME, Imai Y, Kosek J, Yang L, Beal MF, Nishimura I, Wakamatsu K, Ito S, Takahashi R, Lu B (Inactivation of Drosophila DJ-1 leads to impairments of oxidative stress response and phosphatidylinositol 3-kinase/Akt signaling. Proc Natl Acad Sci U S A 102:13670-13675.2005).
Yeragani VK, Tancer M, Chokka P, Baker GB (Arvid Carlsson, and the story of dopamine. Indian J Psychiatry 52:87-88.2010).
Yokota T, Sugawara K, Ito K, Takahashi R, Ariga H, Mizusawa H (Down regulation of DJ-1 enhances cell death by oxidative stress, ER stress, and proteasome inhibition. Biochem Biophys Res Commun 312:1342-1348.2003).
Yoritaka A, Hattori N, Uchida K, Tanaka M, Stadtman ER, Mizuno Y (Immunohistochemical detection of 4-hydroxynonenal protein adducts in Parkinson disease. Proc Natl Acad Sci U S A 93:2696-2701.1996).
Zeevalk GD, Razmpour R, Bernard LP (Glutathione and Parkinson's disease: is this the elephant in the room? Biomed Pharmacother 62:236-249.2008).
Zeng BY, Iravani MM, Jackson MJ, Rose S, Parent A, Jenner P (Morphological changes in serotoninergic neurites in the striatum and globus pallidus in levodopa primed MPTP treated common marmosets with dyskinesia. Neurobiol Dis 40:599-607.2010).
Zhang J, Graham DG, Montine TJ, Ho YS (Enhanced N-methyl-4-phenyl-1,2,3,6-tetrahydropyridine toxicity in mice deficient in CuZn-superoxide dismutase or glutathione peroxidase. J Neuropathol Exp Neurol 59:53-61.2000).
Zhang J, Perry G, Smith MA, Robertson D, Olson SJ, Graham DG, Montine TJ (Parkinson's disease is associated with oxidative damage to cytoplasmic DNA and RNA in substantia nigra neurons. Am J Pathol 154:1423-1429.1999).
Zhang L, Shimoji M, Thomas B, Moore DJ, Yu SW, Marupudi NI, Torp R, Torgner IA, Ottersen OP, Dawson TM, Dawson VL (Mitochondrial localization of the Parkinson's disease related protein DJ-1: implications for pathogenesis. Hum Mol Genet 14:2063-2073.2005).
Zhou FC, Bledsoe S, Murphy J (Serotonergic sprouting is induced by dopamine-lesion in substantia nigra of adult rat brain. Brain Res 556:108-116.1991).
Zhou W, Freed CR (DJ-1 up-regulates glutathione synthesis during oxidative stress and inhibits A53T alpha-synuclein toxicity. J Biol Chem 280:43150-43158.2005).

Zhu XR, Maskri L, Herold C, Bader V, Stichel CC, Gunturkun O, Lubbert H (Non-motor behavioural impairments in parkin-deficient mice. Eur J Neurosci 26:1902-1911.2007).

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 (Mutations in LRRK2 cause autosomal-dominant parkinsonism with pleomorphic pathology. Neuron 44:601-607.2004).

Zurkovsky L, Bychkov E, Tsakem EL, Siedlecki C, Blakely RD, Gurevich EV (Cognitive effects of dopamine depletion in the context of diminished acetylcholine signaling capacity in mice. Dis Model Mech 6:171-183.2013).