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# Surprising behavioral and neurochemical enhancements in mice with combined mutations linked to Parkinson's disease

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ABSTRACT

Parkinson's disease (PD) is the second most common neurodegenerative disorder behind Alzheimer's disease. There are currently no therapies proven to halt or slow the progressive neuronal cell loss in PD. A better understanding of the molecular and cellular causes of PD is needed to develop disease-modifying therapies. PD is an age-dependent disease that causes the progressive death of dopamine-producing neurons in the brain. Loss of substantia nigra dopaminergic neurons results in locomotor symptoms such as slowness of movement, tremor, rigidity and postural instability. Abnormalities in other neurotransmitters, such as serotonin, may also be involved in both the motor and non-motor symptoms of PD. Most cases of PD are sporadic but many families show a Mendelian pattern of inherited Parkinsonism and causative mutations have been identified in genes such as *Parkin*, *DJ-1*, *PINK1*, *alpha-synuclein* and *leucine rich repeat kinase 2* (*LRRK2*). Although the definitive causes of idiopathic PD remain uncertain, the activity of the antioxidant enzyme glutathione peroxidase 1 (Gpx1) is reduced in PD brains and has been shown to be a key determinant of vulnerability to dopaminergic neuron loss in PD animal models. Furthermore, Gpx1 activity decreases with age in human substantia nigra but not rodent substantia nigra. Therefore, we crossed mice deficient for both Parkin and DJ-1 with mice deficient for Gpx1 to test the hypothesis that loss-of-function mutations in Parkin and DJ-1 cause PD by increasing vulnerability to Gpx1 deficiency. Surprisingly, mice lacking Parkin, DJ-1 and Gpx1 have increased striatal dopamine levels in the absence of nigral cell loss compared to wild type, Gpx1$^{-/-}$, and Parkin$^{-/-}$DJ-1$^{-/-}$ mutant mice. Additionally, Parkin$^{-/-}$DJ-1$^{-/-}$ mice exhibit improved rotarod performance and have increased serotonin in the striatum and hippocampus. Stereological analysis indicated that the increased serotonin levels were not due to increased serotonergic projections. The results of our behavioral, neurochemical and immunohistochemical analyses reveal that PD-linked mutations in Parkin and DJ-1 cause dysregulation of neurotransmitter systems beyond the nigrostriatal dopaminergic circuit and that loss-of-function mutations in Parkin and DJ-1 lead to adaptive changes in dopamine and serotonin especially in the context of Gpx1 deficiency.



## Introduction

Parkinson's disease (PD) is the most common neurodegenerative movement disorder and afflicts millions of people worldwide. The severity of the primary clinical symptoms, which include bradykinesia, resting tremor, rigidity, and postural instability, increases over the course of many years. Postmortem examinations reveal a profound and selective loss of dopaminergic neurons in the substantia nigra that project to the caudate and putamen of the dorsal striatum. The loss of dopaminergic innervation of the striatum underlies the primary clinical symptoms, which can be ameliorated with dopaminergic medications. Although the definitive cause of nigral dopamine neuron loss remains unknown, aging is the greatest risk factor for PD, consistent with the increased prevalence of PD in elderly populations. 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), suggesting a potential role for cumulative oxidative stress in PD pathogenesis.

The majority of PD cases are idiopathic with no clear family history of Parkinsonian symptoms. However, genetic linkage studies of families with Mendelian patterns of inherited Parkinsonism have identified causal mutations in several genes (Corti et al., 2011; Dawson et al., 2010; Hattori, 2012; Horowitz and Greenamyre, 2010; Lopez and Sidransky, 2010; Varcin et al., 2012). Among these are the loss-of-function mutations in the *Parkin* and *DJ-1* genes that were the first to be causally linked to recessive Parkinsonism (Bonifati et al., 2003; Kitada et al., 1998). Both Parkin and DJ-1 are widely expressed throughout the brain and other tissues (Kuhn et al., 2004; Shang et al., 2004; Shimura et al., 1999; Stichel et al., 2000; Xie et al., 2009) but it is not obvious why the loss of Parkin or DJ-1 function causes selective

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neurodegeneration and clinical PD symptoms. Presumably, dopaminergic neurons within the nigrostriatal pathway are more susceptible than other cells to both Parkinsonian genetic mutations and to factors that cause idiopathic PD, which remain uncertain. Parkin functions as an E3 ubiquitin ligase (Shimura et al., 2000) and promotes autophagy of dysfunctional mitochondria (Narendra et al., 2008). This suggests an important role for Parkin in preventing the accumulation of damaged mitochondria, which are major cellular sources of free radicals and oxidative stress. 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).

Overexpression of either protein is neuroprotective *in vitro* and *in vivo* (Bian et al., 2012; Hayashi et al., 2009; Junn et al., 2009; Lo Bianco et al., 2004; Ulusoy and Kirik, 2008; Vercammen et al., 2006; Zhou and Freed, 2005). In particular, DJ-1 is protective against various oxidative stresses (Andres-Mateos et al., 2007; Junn et al., 2009; Kim et al., 2005; Menzies et al., 2005; Meulener et al., 2005; Moore et al., 2005; Taira et al., 2004; Yang et al., 2005; Yokota et al., 2003; Zhang et al., 2005) and both proteins localize to mitochondria in cells undergoing oxidative stress (Horowitz and Greenamyre, 2010; Kawajiri et al., 2010; Shulman et al., 2011; Thomas et al., 2011). Cysteine 106 of DJ-1 is unusually sensitive to oxidation and is required both for the neuroprotective effects of DJ-1 and for localization of DJ-1 to mitochondria in response to oxidative stresses (Canet-Aviles et al., 2004; Cookson, 2010; Junn et al., 2009; Kim et al., 2005; Lev et al., 2008; Mullett and Hinkle, 2011). Mutations in the *Parkin* gene lead to impaired mitochondrial respiratory chain function and to increased markers of oxidative stress in humans and genetic animal models (Hauser and Hastings, 2013; Muftuoglu et al., 2004; Palacino et al., 2004; Rodriguez-Navarro et al., 2007; Vincent et al., 2012; Vincow et al., 2013; Vinish et al., 2011). Together, these data suggest that the cellular mechanism by which loss-of-function mutations in Parkin and DJ-1 cause Parkinsonism involves diminished protection against oxidative stress.

Despite the high penetrance of loss-of-function mutations in Parkin and DJ-1 in humans (Bonifati, 2007), similar mutations in mice do not produce the characteristic loss of nigral dopaminergic neurons that is the most prominent postmortem neuropathological feature of PD (Andres-Mateos et al., 2007; Chandran et al., 2008; Fleming and Chesselet, 2006; Fleming et al., 2005; Frank-Cannon et al., 2008; Goldberg et al., 2003, 2005; Itier et al., 2003; Kim et al., 2005; Kitada et al., 2009; Manning-Bog et al., 2007; Palacino et al., 2004; Perez and Palmiter, 2005; Perez et al., 2005; Pham et al., 2010; Rousseaux et al., 2012; Sato et al., 2006; Von Coelln et al., 2004; Yang et al., 2007; Zhu et al., 2007). Thus, within the two-year lifespan of mice, additional stress may be required for Parkin and DJ-1 knockout mice to reproduce the neurodegeneration that occurs in humans. It has been demonstrated that mice deficient for DJ-1 are more susceptible to nigral cell loss induced by mitochondrial toxins and oxidative stressors such as paraquat, rotenone, and 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) (Kim et al., 2005; Manning-Bog et al., 2007; Paterna et al., 2007) and Parkin knockout mice are more susceptible to nigral cell loss induced by chronic exposure to lipopolysaccharide (Frank-Cannon et al., 2008). Compensatory changes in enzymes that protect against oxidative stress could explain the lack of nigrostriatal degeneration in Parkin and DJ-1 knockout mice in the absence of additional stresses (Andres-Mateos et al., 2007; Rodriguez-Navarro et al., 2007). Specifically, DJ-1 knockout mice have an age-dependent increase in both the levels and activity of glutathione peroxidase (Gpx1), but not catalase, the two major enzymes that remove hydrogen peroxide from cells (Andres-Mateos et al., 2007). Parkin knockout mice also have an age-dependent increase in Gpx1 activity as well as decreased levels of reduced glutathione in the midbrain of aged knockout mice (Rodriguez-Navarro et al., 2007). Moreover, Gpx1 knockout mice are more susceptible to MPTP and overexpression of Gpx1 can protect against 6-hydroxydopamine-induced nigral cell loss (Bensadoun et al., 1998; Klivenyi et al., 2000; Ridet et al., 2006; Zhang et al., 2000). These studies suggest that the level of Gpx1 activity is a key determinant of vulnerability to nigral neuron loss in PD animal models and that compensatory increases in Gpx1 activity might explain the absence of nigral cell loss in Parkin and DJ-1 knockout mice. Even in the absence of mutations, Gpx1 activity decreases with age in human substantia nigra (Venkateshappa et al., 2012) but not in rodent substantia nigra (Benzi et al., 1989), which may explain the increased vulnerability to nigral cell loss in humans compared to rodents bearing PD-linked mutations.

To better understand the role of Parkin and DJ-1 loss-of-function mutations in PD pathogenesis and to potentially generate a mouse model that better recapitulates the age-dependent neurochemical, neuropathological and behavioral characteristics of PD, we combined Parkin and DJ-1 loss-of-function mutations and tested them in the context of Gpx1 deficiency on a C57BL/6 mouse genetic background. Mice deficient in all three genes (Parkin$^{-/-}$DJ-1$^{-/-}$Gpx1$^{-/-}$) were viable but did not exhibit age-dependent loss of nigral neurons, decreased striatal dopamine, or motor impairments consistent with PD. Contrary to our expectations, Parkin$^{-/-}$DJ-1$^{-/-}$Gpx1$^{-/-}$ mice had increased striatal dopamine levels while Parkin$^{-/-}$DJ-1$^{-/-}$ mice did not have increased striatal dopamine levels, but showed increased serotonin levels in both the striatum and the hippocampus. Mice with increased serotonin also showed improved rotarod behavior performance and were less “distracted” when performing the rotarod test. These data reveal roles for Parkin and DJ-1 in regulating serotonin levels and potentially compensatory increases in striatal dopamine levels in the absence of Gpx1, Parkin and DJ-1. These surprising behavioral and neurochemical phenotypes expand the apparent functions of Parkin and DJ-1 and suggest that the pathogenic mechanisms of Parkin and DJ-1 mutations may involve dysregulation of dopaminergic and serotonergic neurotransmission.

## Materials and methods

### Animals

Parkin knockout mice and DJ-1 knockout mice were generated as previously described (Goldberg et al., 2003, 2005) and backcrossed to strain C57BL/6 J 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 h. The number of beam breaks was recorded in 5-minute bins as a measure of locomotor activity.