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The function of orthologues of the human Parkinson's disease gene LRRK2 across species: implications for disease modeling in preclinical research

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Abstract

In the period since *LRRK2* (Leucine-Rich Repeat Kinase 2) was identified as a causal gene for late-onset autosomal-dominant parkinsonism, a great deal of work has been aimed at understanding whether the LRRK2 protein might be a druggable target for Parkinson's disease (PD). As part of this effort, animal models have been developed to explore both the normal and the pathophysiological roles of LRRK2. However, LRRK2 is part of a wider family of proteins whose functions in different organisms remain poorly understood. In this review, we compare the information available on biochemical properties of LRRK2 homologues and orthologues from different species from invertebrates (*e.g.*, *Caenorhabditis elegans* and *Drosophila melanogaster*) to mammals. We particularly discuss the mammalian LRRK2 homologue, LRRK1, and those species where there is only a single *lrrk* homologue, discussing examples where each of the LRRK family of proteins has distinct properties as well as those cases where there appear to be functional redundancy. We conclude that uncovering the function of LRRK2 orthologues will help elucidate the key properties of human LRRK2 as well as to improve understanding of the suitability of different animal models for investigation of LRRK2-related PD.

Keywords

LRRK2 homologue; animal models; kinase; GTPase; dopaminergic neurons; neurodegeneration; Preclinical research

INTRODUCTION

Parkinson's disease (PD) is characterized clinically by a range of motor symptoms, including postural instability, resting tremor, bradykinesia, and rigidity as well as non-motor features such as hyposmia [1,2], constipation [3], and mood disturbances [4]. Pathologically, the disease is associated with progressive loss of dopaminergic (DA) neurons in the

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substantia nigra and the formation of fibrillar aggregates called Lewy bodies in surviving neurons throughout the brain [5].

The cause of most cases of PD is not known, leading to the description as a sporadic or idiopathic disease. However, in recent years it has been increasingly appreciated that monogenic forms of PD exist and that cloning the underlying gene mutations can lead to important insights into pathobiology [6,7]. A locus on chromosome 12 (historically termed PARK8) was first linked to familial parkinsonism in 2002 through analysis of a large Japanese family in which affected members exhibited clinical phenotypes resembling those of idiopathic PD [8]. In 2004, late-onset autosomal-dominant parkinsonism was linked to the same locus in multiple families, and a number of pathogenic mutations in the *LRRK2* gene were identified [9–11]. Importantly, subsequent investigations of the genetic contribution to sporadic PD using genome-wide association studies have nominated variation around the wild-type *LRRK2* gene as a risk factor for the common form of this disease [12,13]. Thus, the *LRRK2* gene likely links sporadic and inherited PD.

The *LRRK2* gene encodes a large multi-domain protein, LRRK2. The N-terminal region of LRRK2 comprises a series of repeats predicted to adopt armadillo-like and ankyrin repeat folds as well as a leucine-rich repeat (LRR) domain for which LRRK2 and its homologues are named. The C-terminal region also contains a set of repeats that form a predicted β -sheet rich WD40 domain. These protein interaction motifs flank a catalytic tridomain: a Roc (Ras of complex proteins) GTPase domain in tandem with its associated C-terminal of Roc (COR) domain, followed by a kinase domain [14]. Notably, the COR domain always follows the Roc domain within proteins belonging to the Roco protein family [15] and may play an important role in intramolecular interactions between the two catalytic domains of LRRK2 [16].

As well as LRRK2, there are three other Roco proteins that have been identified in the human proteome, with the closest homologue being leucine-rich repeat kinase 1 (LRRK1), death-associated protein kinase 1 (DAPK1) and malignant fibrous histiocytoma amplified sequences with leucine-rich tandem repeats 1 (MASL1). All four have been found capable of binding guanosine nucleotides via the Roc domain and LRRK2 and DAPK1 are active kinases [17–22], suggesting that there are functional similarities between the human Roco proteins.

These human proteins are part of an evolutionarily older superfamily of Roco proteins [23,24] that include representatives from the slime mold *Dictyostelium discoideum* through most of the animal kingdom, with a few representatives in plants such as the gene TORNADO1 in *Arabidopsis*. The evolutionary history of the modern LRRK proteins can be traced to the cnidarian *Nematostella vectensis*, which has four LRRK genes. Two of these genes are orthologues of the LRRK1 and LRRK2 genes present in deuterostomes (including humans and rodents), while a third is an orthologue of the LRRK gene in protostomes such as *C. elegans* and *Drosophila*. The fourth appears to be cnidarian-specific [24,25]. Thus, the *C. elegans* (Irk-1) and *Drosophila* (Lrrk) genes have an ancient origin distinct from that of LRRK2.

Here, we will focus on LRRK proteins across the animal kingdom, discussing the functional redundancy between these different proteins but also those examples where different proteins seem to have different functions within or between different species. This will also help to frame later discussions of the suitability of different animal models for the study of Parkinson's disease and for preclinical trials of novel therapeutic treatments. For further discussion, including an overview of two studies of zebrafish LRRK2 models, the interested reader may refer to another recent review article about LRRK2 animal models [26]. We will start with two well-characterized invertebrate model organisms, *C. elegans* and *Drosophila melanogaster*.

The *Caenorhabditis elegans* protein, Lrk-1

The transparent nematode *Caenorhabditis elegans* offers a unique model system for neurodegenerative disease as the 302 neurons that constitute the nervous system of an adult *C. elegans* hermaphrodite can be examined *in vivo* and, of particular importance to PD research, the eight dopaminergic neurons can be easily visualized [27–29]. *C. elegans* has a single orthologue of mammalian LRRK genes, *lrk-1*. The Lrk-1 protein is slightly shorter than LRRK2 (2,393 amino acids) but similarly organized with clear LRR, Roc, COR, and kinase domains [28] (Figure 1). Sequence homology between LRRK2 and Lrk-1 is modest, with 24/40% identity/similarity over a region of ~1,500 amino acids [30,31]. As discussed above, because *lrk-1* has a distinct evolutionary history from LRRK1 and LRRK2, this relatively low level of similarity is not surprising and suggests that *lrk-1* knockouts should be considered distinct from knockouts of LRRK2, which we will discuss later in this article.

Worms carrying deletion mutations in *lrk-1* exhibit a range of phenotypes, including subtle motor defects [28], aberrant localization of synaptic vesicle proteins [28], and increased sensitivity to a variety of pharmacological stressors [30–33]. Along with regulation of polarized sorting of synaptic vesicle proteins in neurons [28], *lrk-1* has also been functionally linked to regulation of neurite outgrowth [31]. Evidence that LRRK2 can substitute for *lrk-1* in reducing neurotoxicity [32] and that knockdown of endogenous *lrk-1* and expression of human LRRK2 have reciprocal effects [29,30,33] suggests that *lrk-1* and LRRK2 can interact with the same pathways in *C. elegans*. However, overexpression of either *lrk-1* wild-type or *lrk-1* G1876S (corresponding to the human LRRK2 G2019S mutation) caused embryonic lethality [31], which might imply that the native protein possesses an intrinsic toxicity to nematodes not conserved in the human LRRK2 protein.

Transgenic expression of wild-type human LRRK2 in nematodes was associated with increased lifespan and protection against whole organism toxicity caused by rotenone or paraquat [30,33]. Transgenic worms expressing mutant human LRRK2 were protected against rotenone and paraquat toxicity to a lesser extent than observed in LRRK2 wild-type animals [30,33] and displayed adult-onset dopaminergic neuron loss sometimes accompanied by motor deficits [29,30,32,34,35]. R1441C and G2019S mutants caused a more severe phenotype than wild-type LRRK2, but expression of the GTPase binding defective mutant K1347A did not induce the parkinsonian phenotype [29]. Similarly, there is evidence to support the contribution of kinase activity of LRRK2 mutants in PD pathology [29,35]. Some phenotypes in these animals could be rescued by administration of L-DOPA

[32,34]. However, when examining dopamine neurons directly, G2019S-LRRK2 transgenic nematodes displayed significant loss of dopaminergic markers on adult days 2 and 3 relative to wild-type, both basally and in response to mitochondrial toxins [30]. Interestingly, knockdown of *lrk-1* was found to attenuate the pathogenic effects of LRRK2 expression [29,32], suggesting that overexpression of mutant LRRK2 may work, in part, by a dominant negative effect on the endogenous protein. However, why LRRK2 is protective in some assays but toxic in others is not yet resolved.

Collectively, these results suggest that there may be some shared functions between human LRRK2 and *C. elegans lrk-1*. An important caveat, particularly in relation to the transgenic approaches, is that levels of proteins are not usually assessed. Thus, whether the two proteins can be directly compared or whether there are non-specific events related to overexpression is difficult to determine. Furthermore, the observation that there are effects seen with *lrk-1* not shared with LRRK2 [31] suggests that the two proteins have at least some distinct properties. It would be important in the future to understand the similarities and differences between human LRRK2 and worm *lrk-1* with more mechanistic approaches, particularly at the level of protein interactions or substrates.

The *Drosophila melanogaster* protein, Lrrk

Like *C. elegans*, *Drosophila melanogaster* has a single orthologue of LRRK1/2 named Lrrk, an approximately 2,400 amino acid long protein containing LRR, ROC-COR, and kinase motifs [36,37]. However, Lrrk does not appear to contain the LRRK2-like N-terminal ankyrin repeats or a C-terminal WD40 domain. Many of the residues involved in LRRK2 PD pathology are conserved between human LRRK2 and *Drosophila* Lrrk, which share 24% identity and 38% similarity overall [36–38] (Figure 1). Lrrk is ubiquitously expressed in *Drosophila* tissues, including in fly brain [36,39,40]. Within cells, Lrrk protein is largely cytoplasmic with some association with membranous structures including endosomes, lysosomes, and synaptic vesicles [36,41], thus being broadly similar to human LRRK2.

The Lrrk protein is an active kinase capable of autophosphorylation [36] and of phosphorylation of various proposed substrates, including 4E-BP, Futsch, Endophilin A, and ribosomal protein s15 [39,42,43]. Lrrk has been shown to physically interact with Rab5, Rab7, and Rab9 in *Drosophila* follicle cells [41,44] and to associate with *Drosophila* Argonaute-1 (dAgo1) of the RNA-induced silencing complex in fly head extracts [45]. As human LRRK2 is known to interact with multiple Rab proteins [46–49], and is claimed to phosphorylate some of the same substrates [39,42,43] and to associate with Ago1 [45], there may be at least partial conservation of function between Lrrk and LRRK2. Supporting this hypothesis, several experiments have indicated that Lrrk knockdown and human LRRK2 expression have reciprocal effects [42,43,50] or that overexpression of Lrrk and overexpression of human LRRK2 have the same effect [51]. However, not all phenotypes related to loss of Lrrk have been reproducible. For example, locomotor dysfunction and abnormal morphology of dopaminergic neurons in Lrrk deficient flies from one study [37] were not reproduced in independently generated Lrrk deficient lines [36,38]. Therefore, some aspects of Lrrk function remain to be clarified.

Transgenic expression of mutant Lrrk in *Drosophila* dopaminergic neurons has consistently resulted in age-dependent dopamine cell death. Imai *et al.* reported that 60-day-old but not 10-day-old transgenic flies expressing *Drosophila* Lrrk Y1383C (corresponding to human LRRK2 Y1699C) or I1915T (corresponding to human I2020T mutant) driven by a *TH-Gal4* showed a significant reduction in both number of dopaminergic neurons and dopamine content compared to those expressing wild-type Lrrk [36]. Gehrke *et al.* also observed DA neuron loss as well as climbing defects in *TH-Gal4*-directed pathogenic *Drosophila* Lrrk I1915T and human LRRK2 G2019S transgenic animals at 65 days of age [45].

Similarly, mutant human LRRK2 is toxic in flies. Aged flies expressing *ddc-GAL4*-driven *UAS-G2019S-LRRK2* exhibit selective degeneration of DA neurons, motor dysfunction, and reduced lifespan [35,40,43,51–53]. Linhart *et al.* expressed *ddc-GAL4*-driven mutant human LRRK2 I2020T in transgenic *Drosophila* lines, which also resulted in the loss of dopaminergic neurons, locomotor deficits, and decreased lifespan [54]. Ng *et al.* generated mutant flies with *ddc-Gal4*-directed expression of human LRRK2 Y1699C. These flies were phenotypically similar to flies expressing human LRRK2 G2019S. Interestingly, human LRRK2 G2385R mutant flies did not show significant degeneration of DA neurons or motor deficits, but did have a significantly reduced lifespan [52]. Flies overexpressing mutant Lrrk/LRRK2 were also found to be more vulnerable to hydrogen peroxide (H₂O₂) compared to those overexpressing wild-type protein [36,55], along with decreased sensitivity to the H₂O₂ treatment in *Drosophila* Lrrk deletion mutant flies [36]. However, Wang *et al.* found Lrrk deletion mutant flies to be more sensitive to H₂O₂ than wild-type flies [38]. Expression of wild-type human LRRK2 did not induce a phenotype significantly different from that of control in some studies [35,51–53,56], but a mild phenotype has been reported in others [40,57]. Hindle *et al.* did not see reduction in number of DA neurons in 28-day-old flies expressing human LRRK2 G2019S directed by *TH*- or *HL9*-dopaminergic GAL4 drivers, but did find retinal degeneration [56].

Despite some discrepancies between different transgenic lines, these models have led to several important insights into the pathogenesis of LRRK2-mediated disease. For example, both *Drosophila* Lrrk and human LRRK2 modulate neurite growth and synaptic morphogenesis via phosphorylation of Futsch, the *Drosophila* homolog of microtubule-associated protein 1B (MAP1B), at the pre-synapse and of eukaryotic initiation factor 4E (eIF4E)-binding protein (4E-BP) at the post-synapse [39]. While it remains unclear how 4E-BP, a negative regulator of protein translation, mediates the synaptic effects of LRRK2, this hypothesis would be consistent with prior claims that 4E-BP is a substrate of *Drosophila* Lrrk [36], although data from human cells suggests that the phosphorylation of 4E-BP might be regulated by other stress-induced kinases such as p38 [58]. LRRK2/Lrrk have also been claimed to phosphorylate FoxO, a transcription factor that regulates 4E-BP transcription, potentially suggesting a mechanism other than direct phosphorylation of 4E-BP by LRRK2/Lrrk to control 4E-BP [59]. Additional experiments linked deregulated protein synthesis with increased LRRK2 kinase activity in human LRRK2 G2019S transgenic flies through increased phosphorylation of ribosomal protein s15, an activator of protein synthesis [43]. As yet another potential mechanism, Gehrke *et al.* showed that pathogenic *Drosophila* Lrrk I1915T and human LRRK2 G2019S negatively regulate translational repression via the microRNA pathway [45], although subsequent studies looking at miRNA abundance in

LRRK2 knockout or transgenic mice showed a very modest effect on transcription [60], leaving open the question as to whether the observed effects on translation are a direct consequence of LRRK2 or subsequent to the accumulation of age-related pathology in *Drosophila* Lrrk/LRRK2 models.

Another concept that has been derived largely from fly models is that bioenergetics might be important in Lrrk/LRRK2 pathogenesis. For example, retinal degeneration in flies expressing human LRRK2 G2019S can be prompted by increased activity/energy demand in the visual system [56]. Mitochondria, which are clearly important in energy generation, show pathology in the flight muscles of G2019S–LRRK2 transgenic *Drosophila* [53]. These effects are modulated by both parkin and LRRK2 via the AMPK signaling pathway, further implying that compromised neuronal energy homeostasis could contribute to neurodegeneration [53]. Multiple studies of transgenic LRRK2 *Drosophila* models reported a genetic linkage with parkin [52,53,57], PINK1 [57], or DJ-1 [57], genes that are known to be important in mitochondrial function [61].

An apparently distinct set of interactions between *Drosophila* Lrrk and the Rab protein family in *Drosophila* follicle cells suggest roles for Lrrk in Rab7-dependent lysosomal positioning and in endosomal recycling via Rab9 [41,44]. Matta *et al.* suggested an involvement of *Drosophila* Lrrk in synaptic vesicle endocytosis at the *Drosophila* neuromuscular junction through phosphorylation of Endophilin A, a result later supported by experiments in rodent cells [42,62]. Linhart *et al.* provided evidence of interaction between human LRRK2 and vacuolar protein sorting 35 (Vps35) using a genetic modifier screen with transgenic human LRRK2 I2020T mutant flies [54]. Mutation of the Vps35 gene, which encodes a core component of the retromer complex critically involved in protein sorting and recycling and endosome-trans-Golgi trafficking, has been identified as causative of PD [63,64]. These results would then suggest that one or more aspects of vesicular transport, particularly related to turnover of endosomes, might be impacted by loss of *Drosophila* Lrrk.

Finally, *Drosophila* Lrrk may also have effects on regulation of the cytoskeleton. It has been claimed that human LRRK2 can phosphorylate the microtubule binding protein Futsch, leading to altered synaptic microtubule organization and, hence, synaptic growth [39]. Additionally, human LRRK2 is thought to bind to the *Drosophila* glycogen synthase kinase (GSK3b) homologue, Shaggy, that can then phosphorylate tau [51], which would then also be expected to affect microtubule dynamics.

How do we interpret claims that a single protein is involved in at least four distinct cellular processes, namely protein translation, mitochondrial bioenergetics, vesicular transport and cytoskeletal regulation? Two disparate answers to this question are either that LRRK2 genuinely participates in all of these processes, perhaps playing a coordinating role, or that some effects are secondary to others. For example, autophagy is at least one homeostatic mechanism by which dicer and argonaute are degraded [65], raising the possibility that the effects of LRRK on miRNA generation [45] are secondary to its effects on lysosomal positioning [41]. Similar arguments may be made for the relationship between autophagy and energetics as these two processes are tightly co-regulated and selective autophagy is one

way in which mitochondria are degraded [66]. For these reasons, mechanistic details such as whether claimed Lrrk/LRRK2 substrates can be shown to be reliable are very important as they might help resolve which events are closest to LRRK. A related question is the extent to which we can extrapolate from those organisms that have a single homologue to those that have distinct LRRKs. We will therefore discuss next the relationship between LRRK1 and LRRK2, the two paralogous genes found in mammals.

The mammalian paralogues LRRK1 and LRRK2

LRRK1 has 1,981 amino acids [25], sharing a similar overall domain structure [20,25,67,68] and an overall sequence homology of 26/45% identity/similarity [69] to the larger (2,527 amino acid) LRRK2. Both LRRK1 and LRRK2 bind GDP/GTP with a similar affinity via the GTPase-like Roc domain [20,67,69]. Although the regulatory interplay between the Roc domain and the kinase domain is not yet well understood, LRRK2 kinase function does require an intact Roc domain [16,70,71] whereas GTP-binding was shown to stimulate LRRK1 kinase activity [20]. However, differences between the two proteins exist in both the N- and C- terminal regions as LRRK2 has a larger N-terminal region containing several unique repeats that are absent in the equivalent section of LRRK1 [23,25,67,72,73] (Figure 1).

A reasonable question, therefore, is whether LRRK1 and LRRK2 act in the same pathway or have functionally distinct roles. Both LRRK1 and LRRK2 are widely expressed in the mammalian brain including in the frontal cortex, striatum, and hippocampus [69,74–76] with the overall level of LRRK2 mRNA expression in brain higher than that of LRRK1 [77]. However, studies using *in situ* hybridization detected very low if any LRRK1 expression in striatum and also showed that, unlike LRRK2, LRRK1 is not expressed in all neuronal layers [78,79]. Regarding subcellular distribution, LRRK1 is predominantly cytoplasmic as is LRRK2 [18,20,69]. Because they are co-expressed and co-localized in some areas of the brain, a heterodimeric interaction between LRRK1 and LRRK2 could potentially occur *in vivo* as *in vitro* [73,74,80]. It is therefore possible that LRRK1 could play a modulatory role in the LRRK2 signaling network and hence change risk of PD [73,74,80]. A study by Dachselt *et al.* identified a LRRK1 variant, L416M, that was associated with a 6-year earlier age of onset of PD when carried alongside the LRRK2 G2019S mutation, supporting the idea of LRRK1 as a potential disease modifier [74]. However, attempts to directly link variants in LRRK1 to PD pathogenesis have so far been unsuccessful [68,76,81].

A further development of the concept that LRRK1 and LRRK2 might be in the same pathway is that the two proteins might be functionally redundant [77,82], *i.e.*, that LRRK1 could compensate for lack of LRRK2. Potentially arguing against this possibility are the observations that (i) disturbance of LRRK2 does not alter expression of LRRK1 [83,84], (ii) mutations in LRRK1 do not mimic the pathological effects of the equivalent mutations in LRRK2 [69], and (iii) LRRK2 possesses an inherent toxicity not matched by LRRK1, possibly due to the higher basal level of LRRK2 kinase activity [67,69]. Kinase assays of LRRK2 demonstrate robust autophosphorylation as well as phosphorylation of model substrates including LRRKtide, Nictide, myelin basic protein (MBP), and moesin *in vitro* [67,85]. LRRK1 also binds ATP, but autophosphorylation activity is much more modest [86]

and is inactive against LRRK2 model peptides [67,85]. Therefore, although both LRRK1 and LRRK2 are at least recognizable as kinases they do not have precisely the same enzymatic function.

Though like many other kinases both proteins are basally phosphorylated [69], Reyniers *et al.* ascertained that different residues are phosphorylated in each protein by mapping LRRK1 and LRRK2 phosphosites in parallel [73]. One example is the LRRK2-specific binding of 14-3-3 mediated by phosphoresidues S910/S935 that are not found in LRRK1 [73,87,88]. Each protein also has distinct interaction partners. As well as 14-3-3 proteins, LRRK2 has interactions with a number of pre-synaptic proteins mediated by its WD40 domain [89] that do not appear to be shared with LRRK1. In contrast, LRRK1 specifically binds the adaptor protein Grb2 that has been shown to mediate complex formation between LRRK1 and BCR-ABL1, a fusion tyrosine kinase that causes leukemia [90]. Additionally, LRRK1 plays a role in cell division downstream of Polo-like kinase 1 (PLK1) as mitotic spindle orientation depends on centrosome maturation, which requires LRRK1-mediated phosphorylation of CDK5RAP2 [91]. LRRK1 also has a specific interaction with epidermal growth factor receptor (EGFR) [92] that is not shared with LRRK2 [73]. Finally, a study of *Lrrk1* knockout mice presented evidence that severe osteopetrosis is observed in these mice because *Lrrk1* regulates bone mass through interaction with the cellular Rous sarcoma oncogene (*c-Src*) signaling pathway, whereas *Lrrk2* knockout mice do not display a bone phenotype [93]. These results are substantiated by a high-throughput screening of mouse gene knockouts that identified *Lrrk1* as a gene affecting bone mass [94]. Again, these data support some distinctions between LRRK1 and LRRK2.

At the cellular level, both proteins are cytosolic or associated with membranous and vesicular structures [20,69,92,95–97]. LRRK1 has been proposed to regulate EGFR trafficking in endosomes [73,92] via phosphorylation of its substrate CLIP-170, a microtubule plus-end protein [98]. LRRK2 has also been linked to vesicular trafficking, but to date it appears to modulate distinct vesicular transport events related to vesicular sorting and/or the autophagy/lysosome system [46,54,73], likely mediated by protein interactions at the late endosome or the trans-Golgi network (TGN). So while LRRK1 and LRRK2 are both involved in vesicular events that fundamentally alter protein trafficking, it would initially appear that LRRK1 is directed more to the plasma membrane and early recycling endosomes while LRRK2 is involved in later endosomal events and potentially in retromer function. With this in mind, we will next discuss the attempts to model loss of function and PD-related alleles of LRRK2 in rodents.

Rodent models of LRRK2

The LRRK2 protein is highly conserved between humans, mice and rats, with over 88% identity in the amino acid sequences of each species [99] (Figure 1). *Lrrk2* mRNA and protein are expressed throughout both mouse and rat brain with highest expression levels in cortex and striatum, and an overall distribution pattern similar to that of LRRK2 in the human brain [75,95,99–104]. Much like human LRRK2, mouse and rat *Lrrk2* have been shown to localize in the cytoplasm and also to associate with membranous and vesicular structures within cells [95,97,101,105]. It has recently been confirmed that mouse *Lrrk2* and

human LRRK2 can form a cross-species heterodimer by co-transfection and immunoprecipitation, further supporting that they are functionally similar proteins [106].

There has been some debate as to whether or not Lrrk2 is present in the rodent substantia nigra as some studies did find nigral expression [75,95,101,103,104] and some did not [100,102], an issue which may be due to variation in antibody specificity and sensitivity. West *et al.* saw Lrrk2 expression in the substantia nigra in mice but not in rats and also pointed out that although the amino acid sequence of human and mouse LRRK2 is highly conserved, there are marked differences in the regulatory regions across species. These species-specific elements may lead to important distinctions in how the *LRRK2* gene is expressed in different animals [99]. Because of these potentially important distinctions between how LRRK2 behaves in even relatively closely related species, we will discuss mouse and rat models separately, starting first with knockout models.

Loss of function models

Lrrk2 knockout mice—Because LRRK2 mutations are associated with PD, an important question is whether deletion of the *LRRK2* gene influences neurodegeneration and any attendant behavioral outcomes. Several Lrrk2 knockout mouse models yielded animals that did not show loss of dopaminergic neurons (evaluated from 18–24 months of age) or abnormal locomotive behavior [82,107–110]. Another knockout model also showed no neuronal loss, but had a subtly enhanced motor performance in the rotarod test at 7 months and increased thigmotaxic behavior in the open field test at 7 and 16 months [83]. Collectively, these results suggest that expression of LRRK2 is not required for dopamine neuron survival in the adult brain.

In contrast to the modest effects in the brain, several studies have shown a reproducible kidney pathology in Lrrk2 knockout mice. Dramatic morphological changes in color, size, weight, and texture are reported in conjunction with a range of other observations including altered levels of autophagy markers [82,83,107,110], increased inflammatory response [82,83], indications of oxidative damage [82,110], and evidence of interplay between LRRK2 and α -synuclein [82,110] in kidney. The reason that kidneys are affected is proposed to be that LRRK2 expression is high, and LRRK1 expression is low, in this tissue. The relative expression of the two genes might therefore indicate some partial redundancy in other tissues.

The mechanism by which LRRK2 deficiency results in kidney pathology may relate to the autophagy pathway [111,112]. Observations using light and electron microscopy suggest the accumulation of vesicle-derived structures that might be related to autophagosomes or lysosomes. At the protein level, Tong *et al.* noted increased levels of p62, a ubiquitin-binding protein involved in autophagy, and significantly decreased levels of LC3-II in Lrrk2 knockout mouse kidneys at 20 months of age [82]. In a later study, it was noted that there are age-dependent alterations of these markers, with increased levels of LC3-II and decreased levels of p62 in kidneys of 7-month-old Lrrk2 knockout mice but decreased LC3-II and increased p62 in kidneys of 20-month old Lrrk2 knockout mice [110]. In an independent knockout line, increased levels of p62 and of mTOR (mammalian target of rapamycin), a key regulator of autophagy, were seen but the autophagy marker LC3-II was similar to wild-

type in kidneys of *Lrrk2* knockout mice aged 14 months [107]. Finally, Hinkle *et al.* found no evidence of biphasic changes, but instead observed consistently increased levels of LC3-II and p62 in the kidneys of 3-, 12-, and 18–20-month-old *Lrrk2* knockout mice, suggesting a progressive increase in autophagic activity in the kidneys [83] (Table 1). Therefore, the majority of available evidence suggests some change in autophagy and/or lysosomal markers but the data is still inconsistent as to which changes are seen and the role of aging.

Lrrk2 knockout mice have also been used to highlight a potential role for LRRK2 in the immune system. For example, *Lrrk2* knockout mice are more sensitive than their wild type counterparts to inflammatory colitis induced by dextran sulfate sodium [113]. In contrast, the same animals are less sensitive to another inflammatory condition, experimental autoimmune uveitis [114]. It has been suggested [114] that the difference between these two studies relates to the relative contribution of the adaptive immune system compared to innate immunity, the latter being more important in experimental colitis. Supporting this idea, there is evidence from human systems that LRRK2 is responsive to signaling pathways relevant to innate immunity [88,115]. It is likely that the involvement of LRRK2 in both autophagy and responses to pathogens are related to each other as a form of autophagy, xenophagy, is important in the removal of pathogens. By extension, this may mean that inflammation plays a role in the pathology of human LRRK2-mediated PD (reviewed in [116]). Overall, these studies show that important insights into the normal biological function of *Lrrk2* have been obtained from studies of knockout mice. We will next examine which aspects of function of this protein are conserved in rats.

***Lrrk2* knockout rats**—Similarly to *Lrrk2* knockout mouse models, robust pathophysiological changes were detected in the kidneys of *Lrrk2* knockout rats [117–119]. Gross morphological and histological alterations that appear to be progressive with age [118] were observed, implicating a range of pathogenic mechanisms including impairment of the autophagy-lysosomal pathway [118], disrupted immune function [117,119], and deregulated metabolism [117,118]. Biochemical profiling of *Lrrk2* knockout rat kidneys indicated that their abnormally dark pigmentation is due to accumulation of hemoglobin and lipofuscin [119]. Pathophysiological alterations were also seen in rat lungs [118], liver [118], and spleen [117]. Therefore, although the underlying function of LRRK2 is not known, the function is likely to be highly conserved between rats and mice.

Interestingly, overexpression of human α -synuclein via adeno-associated virus-mediated transduction elicited dopaminergic neurodegeneration in wild-type rats but not in *Lrrk2* knockout rats, suggesting that LRRK2 inhibition could have neuroprotective effects [120] (Table 1). This is consistent with results using inducible expression of human α -synuclein A53T generated in the *Lrrk2* knockout background that showed that LRRK2 expression is required for the toxic effects of α -synuclein [108]. Diminished recruitment of proinflammatory myeloid cells was also observed in the *Lrrk2* knockout rats, which would be consistent with a role of *Lrrk2* in innate immunity leading to neurodegeneration as discussed above..

Models of dominant mutations

LRRK2 R1441C/G mouse models—The most dramatic behavioral phenotype in mice using pathogenic mutations in LRRK2 was generated using a BAC transgenic approach to express human R1441G LRRK2. These mice were hypokinetic beginning at 6 months of age, with motor activity deficits progressing to a visually apparent immobility by 10–13 months [121]. Additionally, the motor deficits could be rescued by treatment with the dopamine precursor *L*-DOPA (L-3,4-dihydroxyphenylalanine) or apomorphine, a non-selective D1 and D2 dopamine agonist. There was no loss of dopaminergic neurons, though the DA neurons had abnormally small cell bodies on average and reduced dendritic density in mice aged 9–10 months [121]. This line can be obtained from The Jackson Laboratory, but subsequent reports were not able to reproduce the originally observed motor dysfunction. Bichler *et al.* saw only subtle motor deficits after the age of 16 months in R1441G mice subjected to open field, rotarod, cylinder, and grip strength tests [122]. Dranka *et al.* measured defects in coordinated motor function by rotarod and pole tests in the mice by 15 months of age, but observed unaltered gross motor function in open field and gait tests. Re-examination of dopaminergic neurons revealed normal tyrosine hydroxylase staining in both substantia nigra and striatum of R1441G mice at 16 months of age [123,124] (Table 1). The reasons for this loss of phenotype are not clear, but could potentially relate either to genetic modifiers in the background of the original animals or a loss of copies of the integrated BAC that would then lead to lower expression levels of human LRRK2. It is likely that lower expression of mutant protein would not result in dramatic phenotypes, as a murine *Lrrk2* R1441C knockin model also did not display dopaminergic neurodegeneration or abnormal spontaneous motor activity in mice up to 2 years of age [125].

LRRK2 G2019S mouse models—Only one mouse model has shown both dopaminergic neurodegeneration and motor dysfunction. Using neuronal-specific expression of human G2019S LRRK2, progressive deterioration of SNpc dopaminergic neurons was observed in mice. Reduced locomotor activity was evident via measurement of spontaneous ambulatory activity in an activity cage and performance of the pole test at 12 months, with a slightly more severe phenotype at 16 months. Furthermore, administration of *L*-DOPA reversed the hypoactive phenotype in 12-month-old G2019S mice [105]. A second group also generated a transgenic mouse line with expression of human LRRK2 G2019S driven by the same promoter. In this line, loss of DA neurons and reduced neurite density of remaining DA neurons were found in mice aged to 19–21 months, but the mice displayed normal locomotor activity at 6 and 15 months [126]. Another mouse model using viral-based expression of human LRRK2 G2019S showed a similar loss of dopaminergic neurons and reduced neurite density but no atypical motor phenotype [86].

However, a series of other G2019S mouse models reported normal dopaminergic neurons but with a range of behavioral abnormalities. One knock-in *Lrrk2* G2019S mouse model exhibited a hyperkinetic phenotype and enhanced motor performance that could be reversed with administration of LRRK2 kinase inhibitors [127]. Expression of human G2019S LRRK2 in the forebrain results in normal performance in the rotarod test but significantly increased ambulatory activity in the open field test in mice [108]. Neither wild-type nor

G2019S LRRK2 BAC transgenic mice aged to 7–8 months performed significantly differently from control non-transgenic animals in beam-crossing, gait, or negative geotaxis tests, but G2019S LRRK2 BAC transgenic mice did show reduced exploratory behavior (increased thigmotaxis) in the open field test [84]. BAC transgenic mice overexpressing murine *Lrrk2* G2019S demonstrated normal locomotor activity in the open field, beam and gait tests at 6 and 12 months, but mice overexpressing murine *Lrrk2* wild-type displayed hyperactivity and enhanced performance in these tests [128] (Table 1).

Although these studies have generally focused on the dopamine system, there are some reported abnormalities that could represent symptoms of disease preceding overt neuropathology. Several groups identified signs of altered dopamine neurotransmission [84,105,121,125,128,129], irregular immune response [86,123,124], and/or tau alterations [84,105,121,128]. Gastrointestinal dysfunctions beginning at 6 months of age [122] and olfactory impairment at 15 months of age [123,124] were observed in LRRK2 mice, although not all groups that assessed olfaction ability in LRRK2 mice saw a deficit [122,129]. How these findings may be relevant to human Parkinson's disease patients is not yet clear; regardless, they could indicate promising new strategies for understanding and targeting disease mechanisms.

LRRK2 I2020T mouse model—In the only I2020T mouse model reported in the literature, human LRRK2 I2020T is expressed specifically in neurons [129]. No loss of dopaminergic neurons was observed in either young (10 weeks of age) or aged (18 months of age) mice, but the mice had an abnormal locomotor phenotype. A higher frequency of rearing was seen in 22-week-old transgenic human LRRK2 I2020T mice compared to non-transgenic controls. Additionally, transgenic mice subjected to the beam test (at 23 and 43 weeks of age) and the rotarod test (at 34 weeks of age) exhibited impaired locomotor ability. However, the phenotype did not persist in older animals as no significant difference was observed between transgenic and non-transgenic mice in the performance of the beam test at 73 weeks of age, or the rotarod test at 42 and 59 weeks of age [129] (Table 1).

LRRK2 G2019S rat models—Several models involving overexpression of human LRRK2 G2019S in rats have been described in the literature. Dusonchet *et al.* (2011) instigated neuron-specific expression of human wild-type or G2019S LRRK2 via adenoviral vector injection in the striatum of adult rats. Expression of wild-type LRRK2 did not induce significant neuronal loss in the rats, but animals overexpressing the G2019S LRRK2 mutant displayed progressive degeneration of nigral dopaminergic neurons up to 42 days after injection [130]. Temporal overexpression of human G2019S LRRK2 in 5-month-old inducible transgenic rats did not cause loss of DA neurons, though it did promote enhanced locomotor activity in the open field test in rats at 18 months of age. In contrast, animals constitutively overexpressing human LRRK2 G2019S mutant did not have an atypical behavioral phenotype, which may be indicative of an as yet undefined developmental compensatory mechanism [131]. In a human BAC LRRK2 G2019S transgenic rat model, there was no loss of DA neurons but the neuron cell bodies were significantly flatter than those in wild-type rats at 12 months of age. In a test of postural instability, these human LRRK2 G2019S transgenic rats showed increased instability compared to non-transgenic

littermate controls at 8 months of age, but not at 4 or 12 months, and an increased number of rearings in a cylinder test at 12 months of age, but not at 4 or 8 months [132]. When coupled with adeno-associated viral-mediated overexpression of human α -synuclein, transgenic overexpression of human LRRK2 G2019S in rats induced exacerbated DA neurodegeneration as well as increased microglial activation and myeloid cell recruitment compared with wild-type rats [133]. LRRK2 kinase inhibition mitigated both neurodegeneration and neuroinflammation, supporting the proposal of LRRK2 as a modulator of neuroinflammatory response. Overall, these results show that in rats as in mice, the chronic expression of LRRK2 has relatively modest effects. However, the acute expression from viral vectors in rats, which might be accompanied by modest disruption of the brain parenchyma and transient inflammation, does result in a stronger phenotype.

LRRK2 animal models as a bridge from bench to bedside

The ultimate goal of the extensive research into LRRK2 biology is to develop targeted therapy for people living with PD. To date, most efforts in drug development for LRRK2-associated PD have related to the design of specific, potent, and brain permeable LRRK2 kinase inhibitors. Like any kinase inhibitor, no LRRK2 inhibitor has perfect selectivity and many are only poorly permeable across the blood-brain barrier [134]. Furthermore, recent results have suggested that LRRK2 kinase inhibitors can have effects on other organ systems that may produce detrimental effects [135]. Assuming that these technical concerns can be overcome, kinase inhibitors might be an appropriate treatment option for patients with the G2019S mutation as it is well established that G2019S LRRK2 has an increased kinase activity *in vitro*. However, other LRRK2 mutations apparently have kinase activity similar to wild type protein or even diminished phosphorylation activity [136,137] and therefore may require alternative pharmacological approaches [134]. For example, mutations in the ROC domain (R1441C/G/H) may be targeted with compounds that interfere with GTP binding [138,139]. Importantly for the current discussion, the optimal choice of animal models for each treatment modality stratified by pathogenic mutation would allow estimating *in vivo* efficacy of the drugs as well as revealing potential side effects more effectively during preclinical research prior to human trials.

It is important to be clear what one might expect from an optimal animal for LRRK2. In humans, it is clear that the consistent phenotype associated with mutations is progressive dopaminergic neuronal cell loss in the substantia nigra pars compacta with more variable α -synuclein pathology [140]. Therefore, an ideal animal model would replicate the biochemical effect of each mutation and have dopamine cell loss so that the mutations can be tied to a pathology that has face validity for the human condition. Unfortunately, this crucial phenotype has generally been lacking in many animal models of genetic forms of PD (see [141] for review). One way to provide a stronger effect on dopamine cell loss would be by addition of stressors such as α -synuclein as reported recently in rats [133]. Alternatively, it might be possible to examine intermediate phenotypes, likely autophagy or inflammation, that while not sufficient to induce toxicity in the lifetime of rodents might faithfully report the activity of LRRK2 *in vivo*.

CONCLUSIONS

This survey of the currently available literature has covered concepts related to distinctness and commonality for LRRK2 and its homologues within and between species. Our overall impression at this stage is that while LRRK1 and LRRK2 have importantly different properties they are likely involved in related processes in the cell. An analogy would be the larger family of Rab proteins – all have a similar biochemical function but due to targeting to different subcellular (and in some cases sub-organellar) compartments they can each have different effects in cells. In the case of LRRK1 and LRRK2, it is likely that each might impact similar pathways but in different mechanistic ways. We would therefore predict that knocking out both homologues in tissues would have additive effects rather than demonstrating epistasis and might result in phenotypes that overlap more with those seen in the invertebrate *lrrk-1/lrrk* knockouts than with single LRRK1 or LRRK2 knockouts. The orthologues from invertebrate species may subsume functions of both LRRK1 and LRRK2 in mammals.

Collectively these models have therefore produced substantial and consistent insights into the normal physiological functions of LRRK2 and its homologues across species. A major current focus is on the effects of LRRK2 on the autophagy-lysosome system, but these proteins are also likely involved in cytoskeletal regulation, mitochondrial function and protein translation. It is our view that each of these processes are likely related to each other either directly or indirectly.

All mutations in LRRK2 that cause PD in humans have the ability to produce toxic effects, which has been consistently shown in *Drosophila* models in particular. It is very likely that the toxic effects of mutant LRRK2 relate to its normal biochemical and cellular function(s), particularly to kinase activity. However, mutations do not appear to be simple loss of function as the knockout and mutation phenotypes differ dramatically, especially in vertebrate models. This leads to the most likely current interpretation that mutations are gain of function in some way that remains to be fully defined. Overall, the utility of animal models to dissect mechanisms related to human mutations is easily demonstrated by studies on LRRK2 and it is hoped that this will be useful in the development of therapeutic approaches in the near future.

Abbreviations

BAC	bacterial artificial chromosome
BBB	blood-brain barrier
COR	C-terminus of ROC
DA	dopaminergic
EGFR	epidermal growth factor receptor
L-DOPA	L-3,4-dihydroxyphenylalanine
LRR	leucine rich repeat

LRRK	leucine rich repeat kinase
lrk-1	leucine-rich repeats-1, Ras-like domain, kinase
PD	Parkinson's disease
ROC	Ras of complex proteins
SNpc	substantia nigra pars compacta

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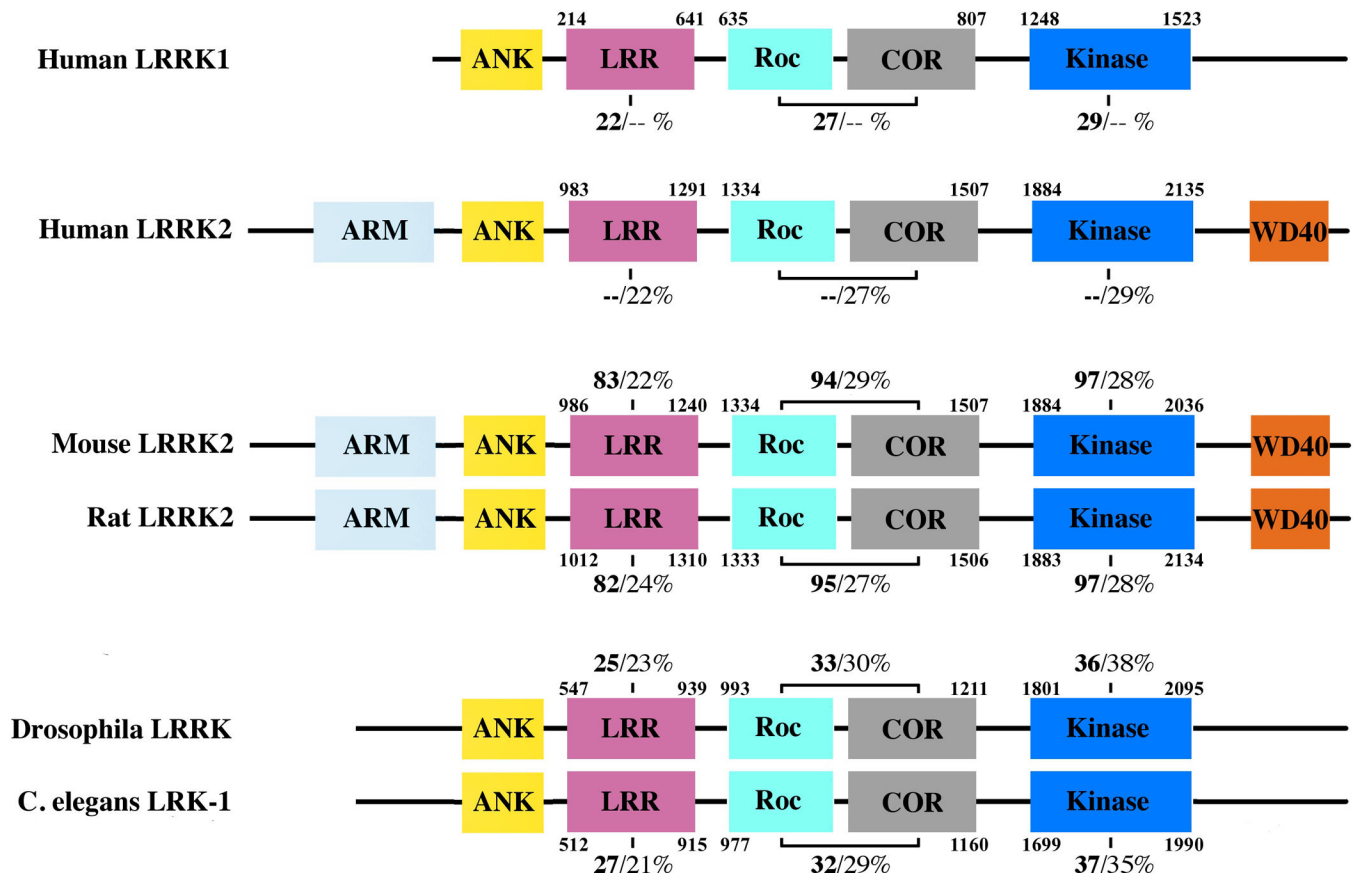


Figure 1.

Schematic diagrams of LRRK proteins in different species, including human LRRK2 and its human homologue LRRK1, as well as orthologues from different species. Predicted domains are indicated by labelled boxes. Percentage identities based on a Clustal 2.1 multiple sequence alignment are given relative to human LRRK2 (left, bolded) and relative to human LRRK1 (right) for LRR, Roc-COR and kinase domains.

Table 1

Rodent models organized by group and characterized by genetic construction, expression level of LRRK2 protein, and the presence, +, or absence, —, of neurodegeneration and/or behavioral phenotypes.

	REFERENCE	GENETIC CONSTRUCT	LRRK2 PROTEIN EXPRESSION LEVEL	PHENOTYPE		
				NEURODEGENERATION	MOTOR ACTIVITY	
MOUSE MODELS	Li 2010	BAC LRRK2 WT	x 6	—	↑↑	
		BAC LRRK2 G2019S	x 6	—	—	
	Herzig 2011	Lrrk2 KO	—	—	—	—
		Lrrk2 KI G2019S [◆]	x 1	—	—	—
	Longo 2014	Lrrk2 KI G2019S [◆]	x 1	—	—	↑↑
		BAC LRRK2 WT	x 3.5	—	—	— (with increased thigmotaxis)
	Melrose 2010	BAC LRRK2 G2019S [■]	x 2.5	—	—	—
		BAC LRRK2 G2019S [■]	NR	NR	NR	NR
	Winner 2010	HSV-LRRK2 G2019S	NR	+++	NR	NR
	Lee 2010	HSV-LRRK2 G2019S	NR	NR	+++	NR
		<i>zet</i> /O-LRRK2 G2019S/CamKII- α TA	x 8-16	—	—	↑↑
	Lin 2009	Lrrk2 KO	—	—	—	—
		CMVE-PDGF β -LRRK2 G2019S	NR	NR	+++	↓↓ (+)
	Chen 2012	CMVE-PDGF β -LRRK2 G2019S	x 3-5	+	—	—
		CMVE-PDGF β -LRRK2 R1441C	x 3-5	—	—	↓
	Ramonet 2011	BAC LRRK2 R1441G'	x 5-10	—	—	↓↓ (+)
		BAC LRRK2 R1441G'	NR	NR	NR	↓
	Li 2009	BAC LRRK2 R1441G'	NR	NR	—	↓
	Bichler 2013	BAC LRRK2 R1441G'	NR	NR	NR	↓
	Dranka 2013	BAC LRRK2 R1441G'	NR	NR	NR	↓
Dranka 2014	BAC LRRK2 R1441G'	NR	NR	NR	↓	
Tong 2009	Lrrk2 KI R1441C	x 1	—	—	—	
Maekawa 2012	CMV-LRRK2 I2020T	x 1.3	—	—	↑↑	
Andres-Mateos 2009	Lrrk2 KO	—	—	—	—	

	REFERENCE	GENETIC CONSTRUCT	LRRK2 PROTEIN EXPRESSION LEVEL	PHENOTYPE	
				NEURODEGENERATION	MOTOR ACTIVITY
RAT MODELS	Tong 2010	Lrrk2 KO*	—	—	NR
	Tong 2012	Lrrk2 KO*	—	NR	NR
	Hinkle 2012	Lrrk2 KO	—	—	↑ (with increased thigmotaxis)
	Ness 2013	Lrrk2 KO [†]	—	NR	NR
	Baptista 2013	Lrrk2 KO [†]	—	NR	NR
	Dusonchet 2011	rAd-LRRK2 WT	x 2	—	NR
		rAd-LRRK2 G2019S	x 2	+	NR
	Zhou 2011	rTA-LRRK2 G2019S; constitutive expression	NR	—	—
		rTA-LRRK2 G2019S; temporal expression	NR	—	↑
	Lee 2015	BAC LRRK2 G2019S	x 5-8	—	↓↑
	Daher 2014	Lrrk2 KO +rAAV α-synuclein	—	—	NR
		wild-type +rAAV α-synuclein	x 1	+	NR
	Daher 2015	wild-type +rAAV α-synuclein	x 1	+	NR
		BAC LRRK2 G2019S +rAAV α-synuclein	x 20-30	++	NR

Superscript symbols (◆, ■, ○, * and †) are used to denote rodents from the same line. Neurodegeneration is defined here as loss of TH+ cells. Up or down arrows are used to indicate an observed increase or decrease, respectively, in motor activity. (+) = phenotype reversed by administration of L-DOPA. NR = not reported.