Published in final edited form as:
Exp Neurol. 2008 January ; 209(1): 22–27.

In vivo alpha-synuclein overexpression in rodents: a useful model of Parkinson's disease?

Marie-Francoise Chesselet, MD, PhD
Departments of Neurology and Neurobiology, David Geffen School of Medicine, UCLA, 710 Westwood Plaza, Los Angeles, CA90095, USA, P: (310) 267-1781, F: (310) 267-1786, E: mchesselet@mednet.ucla.edu

Abstract

Mutations in alpha-synuclein were the first genetic defect linked to Parkinson's disease (PD). The relevance of alpha-synuclein to sporadic PD is strongly supported by the presence of alpha-synuclein aggregates in neurons of patients. This has prompted the development of numerous animal models based on alpha-synuclein overexpression, primarily through genetic methods in mice and viral transduction in rats. In mice, different promoters and transgenes lead to a wide variety of phenotypes accompanied by non-existent, late onset, or non-specific neurodegeneration. Rapid neurodegeneration, in contrast, is observed after viral transduction but is limited to the targeted region and does not mimic the broad pathology observed in the disease. Overall, each model reproduces a subset of features of PD and can be used to identify therapeutic targets and test disease-modifying therapies. The predictive value of all models of the disease, however, remains speculative in the absence of effective neuroprotective treatments for PD in humans.

Why do we need new models of Parkinson's disease?

Animal models of Parkinson's disease (PD) are essential tools to identify novel therapeutic targets and test potential therapies. Since the identification of the loss of nigrostriatal dopaminergic neurons as a main pathological feature of PD, the field has been dominated by toxin-based models, in which a neurotoxin is administered either peripherally or locally to destroy nigrostriatal neurons (Dauer and Przedborski, 2003). These models have had enormous value in helping to understand the consequences of nigrostriatal dopaminergic cell loss, to test symptomatic therapies (dopamine replacement or others), and to identify interventions that reduce lesion size (Bove et al. 2005). The value of these neuroprotective approaches, however, is based on the assumption that the mechanism of action of these toxins is germane to the pathophysiological process occurring in PD. Despite some similarities, and evidence that some of these toxins cause dopamine cell loss in humans (MPTP: Langston et al. 1983) or increase PD risk after long-term exposure (paraquat, maneb: Li W. et al., 2005), there is no clear evidence that the mechanisms are the same. Furthermore, there is no evidence to date that effective neuroprotection against these toxins translates into an effective neuroprotective therapy in humans with PD.

Another major limitation of the toxin-based models is that they do not reproduce the pathology and cell loss observed in other brain regions and peripheral tissues of patients (Halliday et al. 2006), nor the broad range of non-motor symptoms seen in PD (Ziemssen and Reichmann, 2007). Numerous new models of PD based on genetic mutations that cause rare familial forms

of the disease have been generated over the last ten years (Melrose et al. 2006). Because none of theses models is a perfect replicate of the PD pathology and symptoms observed in humans, they are often dismissed as inappropriate tools for drug development and preclinical testing. While it is important to consider their limitations, neglecting the positive attributes of available genetic models slows progress towards new therapies. This is particularly unfortunate at a time when a better understanding of pathophysiological mechanisms of PD is emerging from the genetic findings. The goal of this review is to evaluate the benefits of existing models based on alpha-synuclein overexpression in mice and rats and to highlight their potential for moving the field forward towards a neuroprotective treatment for PD.

Why alpha-synuclein?

In principle, models based on mutations causing familial forms of PD should better mimic the disease (Fleming et al. 2005). One concern, however, is the relevance of the pathophysiological mechanisms triggered by these rare mutations to the much more prevalent sporadic cases. The only mutation to date for which good evidence exists in favor of a link with sporadic PD is overexpression of wild-type alpha synuclein. Indeed, although point mutations in alpha-synuclein can also cause PD, increased levels of the protein due to gene duplication or triplication lead to familial forms of PD (Lee and Trojanowski, 2006). Furthermore, patients with sporadic forms of the disease present with abnormal alpha-synuclein accumulation and aggregates in a subset of central and peripheral neurons (Halliday et al. 2006). Although the cause of alpha-synuclein pathology in the absence of genetic mutations remains unknown, these observations point to a potential role for abnormal forms of the protein in sporadic PD. Which forms are pathological, and even whether alpha-synuclein plays a causal role in the pathology or is simply a “bystander” is not known. However, the genetic data do suggest a causal role for elevated levels of the protein and present a compelling case for modeling the disease by way of over-expressing alpha-synuclein.

Overexpression of alpha-synuclein in transgenic mice

Transgenic mice are easy to use once they are generated, as long as the mutation does not impair breeding or survival. Many different lines of mice over-expressing alpha-synuclein have been generated in the last ten years and most have been described in recent reviews (Fernagut and Chesselet, 2004; Fleming and Chesselet, 2006; Springer and Kahle 2006). Several points need to be considered when assessing their value for evaluating new therapies: how similar is the transgene and its pattern of expression to what is observed in sporadic PD?; how close is the phenotype of the mouse to that of the human disease?; how suitable is the phenotype for preclinical drug testing?; is the model predictive of drug efficacy in patients?

Transgenes and promoters: how well do they mimic sporadic PD?

As indicated earlier, nigrostriatal degeneration, although primarily responsible for the main clinical symptoms of PD, is only one aspect of the disease. Given that pathology in PD includes, and may even start, in other brain regions, models should mimic the broad but regionally selective alpha-synuclein pathology observed in patients (Halliday et al. 2006). Obviously, this cannot be achieved by using a promoter with an expression restricted to catecholaminergic neurons, such as the tyrosine hydroxylase promoter. Nevertheless, several mice generated with this promoter have proven useful. In particular, one of the few models to date that shows apparent nigrostriatal neurodegeneration (assessed only as a loss of tyrosine hydroxylase immunoreactive cell bodies in the substantia nigra, which does not absolutely indicate cell loss) is based on the expression of a doubly mutated alpha-synuclein under the tyrosine hydroxylase promoter (Thiruchelvam et al. 2004). Unfortunately, it is not clear why the double mutation increase alpha-synuclein toxicity. More work on the effect of the double mutation on protein conformation and its ability to form toxic oligomeric forms and/or interact with other cellular

components (Volles and Lansbury, 2003) will be necessary to answer this question. Similarly, expression of truncated alpha-synuclein under the tyrosine-hydroxylase promoter led to nigrostriatal pathology (Tofaris et al. 2006; Wakamatsu et al. 2006). Expression of amino acids 1–130 of the human protein with the A53T mutation caused embryonic loss of dopaminergic neurons in the substantia nigra pars compacta whereas expression of the full length protein did not (Wakamatsu et al. 2006); in another line, expression of amino acids 1–120 of the wild type human protein on a alpha-synuclein null background only led to decreased striatal dopamine without loss of nigrostriatal cell bodies despite the presence of fibrillar alpha-synuclein inclusions (Tofaris et al. 2006). Phosphorylation at serine 129 has been shown to increase pathology in flies (Chen and Feany, 2005). Therefore, the loss of this phosphorylation site may have decreased the toxicity of alpha-synuclein 1–120; however, phosphorylation at that site in the full-length protein is not sufficient to cause neurodegeneration (Wakamatsu et al. 2007). Surprisingly, despite its expression in locus ceoruleus neurons, truncated alpha-synuclein did not cause degeneration of these neurons, which are affected early in the course of the disease in PD patients (Halliday et al. 2006). A key information that emerges from these studies is that some modifications, either double mutation or truncation of the protein, are necessary to obtain cell loss and/or decrease dopamine levels in a mouse. Indeed, numerous lines of mice expressing either wild type or singly mutated alpha-synuclein under the tyrosine hydroxylase promoter have failed to produce these phenotypes (Fernagut and Chesselet, 2004). This may be due to the effects of mutations and truncations of the protein on its ability to form toxic fibrillar species.

These data indicate that even localized expression of various forms of alpha-synuclein in catecholaminergic neurons can provide important information on potential mechanisms of toxicity. What can we conclude of the usefulness of these models for drug development at this point? Differences in the toxicity of different forms of alpha-synuclein in vivo point to characteristics of the protein that are important for pathology and could lead to therapeutic interventions. For example, blocking the formation of fibrils may be a good target if the doubly mutated protein is more fibrillogenic; similarly, if truncation increases toxicity, then it becomes important to identify the proteases responsible for the cleavage of alpha-synuclein in vivo. However, the relevance of these mechanisms of alpha-synuclein toxicity to sporadic PD remains unclear. Patients do not have doubly mutated alpha-synuclein and the phenotype induced by the truncated protein so far does not mimic that of PD: either neuronal loss exists but occurs very early and is not progressive, or does not occur at the time points examined. Therefore, the predictive value of these models for drug efficacy in PD is unproven. With this caveat in mind, they remain potentially very useful because there is a reasonable likelihood that they share mechanisms that occur in sporadic PD. Indeed, mutations accelerate the formation of abnormal forms of the protein that can also be adopted by wild-type alpha-synuclein, and truncated forms of the protein are found in patient brains (Li A. et al. 2005; Follmer et al. 2007).

Other promoters have different advantages and limitations. Many lines of transgenic mice were generated using the prion promoter because it drives high levels of transgene expression in neurons. Although not always the case (Gispert et al. 2003), high level of expression in motoneurons with this promoter often leads to motoneuron pathology, which is not a main feature of PD (Fernagut and Chesselet, 2004). Accordingly, these mice are not useful to evaluate mechanisms of selective neuronal vulnerability in PD but they provide a model to assess general mechanisms of alpha synuclein-induced cell death in vivo, which until recently has been difficult to obtain in nigrostriatal dopaminergic neurons, as indicated earlier (Litvan et al. 2007).

Other promoters used in several lines of mice are the PDGFbeta and the Thy1 promoters. Both confer broad expression of alpha-synuclein in neurons but with different patterns of expression

(Rockenstein et al. 2002). One line with the PDGFbeta promoter develops dopaminergic deficits in the striatum and has been used to demonstrate a neuroprotective effect of antibodies directed anti alpha-synuclein in vivo, a protective effect of virally expressed beta-synuclein and of anti-cholesterol compounds, as well as an enhancing effect of beta-amyloid over-expression (Masliah et al. 2005; Hashimoto et al 2004; Klucken et al. 2004; Hashimoto et al. 2001; Masliah et al. 2001). Thus, these mice proved to be useful for identifying both improvement and worsening of alpha-synuclein toxicity in vivo. The Thy1 lines have a wide range of phenotypes with some lines developing motoneurons pathology (van der Putten et al. 2000) whereas others do not (Rockenstein et al. 2002). This may be related to the use of alpha-synuclein with the A53T mutation, the background stain, level of expression, and/or the insertion site.

Some lines of transgenic mice seem to have been abandoned after initial characterization because mice that do not develop frank nigrostriatal neurodegeneration are often deemed of little value in modeling PD. Mice over-expressing wild-type alpha-synuclein under the Thy1 promoter developed by E. Masliah, however have been extensively characterized. In these mice, the Thy1 promoter confers widespread, high levels of wild-type human alpha-synuclein overexpression in cortical and subcortical neurons, including high levels in the substantia nigra pars compacta, without glial, spinal, or neuromuscular pathology (Rockenstein et al. 2002). Proteinase K-resistant inclusions of alpha-synuclein, a hallmark of alpha-synuclein pathology in PD brains (Neumann et al. 2004) that are also found in other mouse models (Freichel et al. 2007) are detected in many subcortical regions of these mice, including olfactory bulb, substantia nigra, and locus coeruleus (Fernagut et al. 2007; Hutson and Chesselet, unpublished observations), all regions showing alpha-synuclein pathology at early stages of PD (Halliday et al. 2006). These mice show increased mitochondrial pathology when exposed to low doses of MPTP (Song et al. 2004). Despite a minor but significant loss of ligand binding to dopamine cytoplasmic and vesicular transporters in the striatum, they do not show nigrostriatal cell loss up to 8 months of age (Rockenstein et al. 2002; Fernagut and Chesselet, unpublished observations).

An intriguing feature of these mice is the presence of progressive sensorimotor anomalies in tests that also detect deficits in mouse models of nigrostriatal degeneration (Fleming et al. 2004). The alpha-synuclein over-expressors, however, have a full complement of striatal dopamine terminals at a time when they show deficits in these tests, raising the question of the mechanisms leading to the behavioral anomalies. Not surprisingly, direct and indirect dopaminergic agonists do not reverse and even worsen the deficits in these mice, in agreement with evidence for the detrimental effects of excess dopamine (Fleming et al. 2006a). On the other hand, electrophysiological studies have shown profound synaptic anomalies in the striatum of these mice, in particular a marked decreased of spontaneous excitatory potential at the cortico-striatal synapse and abnormal responses to drugs acting at dopaminergic receptors (Wu et al, 2005). In addition, preliminary data indicate a decreased level of noradrenalin in the cortex, even though dopamine levels remain normal in the striatum (Maidment and Chesselet, unpublished observations). Thus, these mice provide a means to unravel the system-wide effects of alpha-synuclein over-expression that may occur at very early stages of PD, before dopaminergic cell loss sets in. Furthermore, these mice reproduce some of the early non-motor symptoms observed in PD, in particular olfactory deficits, and digestive dysfunction (Fleming et al. 2006b; Wang et al. 2005). It remains to be seen whether sleep, affective, and cognitive symptoms of PD can also be observed in these mice. Already, a combination of motor and non-motor end points provides a means to test drugs that could interrupt the effects of alpha-synuclein overexpression before dopaminergic degeneration. Furthermore, the effects of these compounds on insoluble alpha-synuclein aggregates in the brain can be evaluated with biochemical and histological methods.

In summary, although none is a perfect replica of the disease, the existing genetic models of alpha-synuclein overexpression in mice offer a range of possibilities for drug development and testing. Some models that rely upon high levels of expression, mutations, and truncation to accelerate alpha-synuclein pathology may exhibit either progressive or embryonic loss of nigrostriatal neurons (Thiruchelvam et. al. 2004; Wakamatsu et al. 2006). With promoters that lead to high levels of expression in neurons that are not primarily affected in PD, the resulting animals are not good models of selective vulnerability, and their behavioral deficits may not reflect PD symptoms (Fernagut and Chesselet, 2004). However, these animals can inform about general mechanism of toxicity in vivo and their aggressive phenotype may be an advantage for rapid drug testing. Finally models that reproduce more faithfully the pattern of alpha-synuclein overexpression observed in humans tell us that alterations in neuronal circuits not limited to the dopaminergic neurons could occur at early stages of the disease (Levine et al. 2004). This is extremely useful information as it points to mechanisms “upstream” of neuronal death for new therapeutic intervention. Furthermore, the ability to model non-motor PD symptoms in these mice offers interesting end-points for preclinical drug studies and a model in which to study the elusive mechanisms of these deficits (Fleming et al. 2006b). Thus, despite their obvious limitations, even models without overt cell death can greatly benefit studies towards drug development for PD, and the observation of tyrosine hydroxylase positive cells in the substantia nigra of at least one mouse model offers the possibility of assessing neuroprotection per se (Thiruchelvam et al. 2004). Rather than waiting for a perfected model, available mouse lines are worth exploring to identify compounds that may interfere with early stages of the pathological process.

Where do we go from here?

No matter how informative existing models might be they can and should be improved. One clear limitation is the use of promoters that do not reproduce the endogenous pattern of expression of alpha-synuclein in brain. This is currently addressed in several laboratories by generating BAC transgenics with the full alpha-synuclein gene, including its own promoter. Another avenue worth pursuing is the use of different background strains. Most models so far have been made in C57Bl6 mice or on a mixed background including this strain, because these show high sensitivity to nigrostriatal neurodegeneration induced by MPTP and are amenable to a wide range of behavioral testing (Jackson-Lewis and Przedborski, 2007). Other strains, however, have shown sensitivity to neurodegeneration and this could be explored for modeling PD (McLin et al. 2006). Finally, the genetic and/or environment of the mice could be modified to increase alpha-synuclein toxicity in a disease-relevant manner. Changes in patterns of gene expression suggest that over-expression of alpha-synuclein since conception alters genes important for the dopaminergic phenotype (Miller et al. 2007) but also triggers defense mechanisms that may explain their resistance to alpha-synuclein neurotoxicity (Mortazavi et al. 2007). Over-expressing alpha-synuclein in a mouse lacking an essential protective gene may lead to a more complete reproduction of the PD phenotype. These defense mechanisms may also explain why exposure of alpha-synuclein overexpressing mice to environmental toxins known to kill dopaminergic neurons has met with mixed results (Fernagut et al. 2007). However, it is possible that modifying the diet or exposing the mice to sub-threshold environmental insults such as infections or pesticides very early in life would lead to dopaminergic cell loss in a greater number of models (Thiruchelvam et al. 2004). This is worth exploring because a diet low in antioxidants and exposure to common pathogens or environmental toxins would more closely mimic the normal living conditions of humans compared to the optimal housing conditions of laboratory animals. Finally, it will be informative to have models in which alpha-synuclein over-expression can be manipulated by being turned on and off or at different time and/or in different brain regions.

Overexpression of alpha-synuclein with viral vectors

The use of viral vectors to over-express alpha-synuclein represents an important way to model PD in rodents because this approach produces a rapid degeneration of nigrostriatal neurons, a feat not yet reproduced by genetic mutations in mice or rats (Kirik and Bjorklund, 2003). Furthermore, viral gene delivery revealed the ability of wild-type alpha-synuclein to induce nigrostriatal pathology (Kirik et al. 2002), a finding in agreement with evidence in familial forms of PD (Lee and Trojanowski, 2006) and in genetic mouse models (Fernagut and Chesselet, 2004). Several types of viral vectors have been used, primarily lentiviruses and adeno-associated viruses (Kirik et al. 2002; Lo Bianco et al. 2002; Klein et al. 2002; Lauwers et al. 2003; Mochizuki et al. 2006; St Martin et al. 2007). Because viral vector delivery requires stereotactic injections within or near the site of the neuronal cell bodies in the substantia nigra pars compacta, rats are generally used, although the model has been reproduced in mice (St Martin et al. 2007). The relative merits of the various viral vectors are beyond the scope of this review, and this section will be limited to general comments on the advantages and limitations of this approach for modeling PD compared to other models.

A major drawback of gene transfer is the labor-intensive nature of the stereotactic injections and the need to test each batch of virus to insure reproducibility of the results. The need for expertise in virology also restricts the application of this technique to few laboratories. Improper handling may lead to poor reproducibility, and decrease investigator's confidence in the validity of this model. This should be readily addressable with proper training. Apparent lack of reproducibility of the technique may also be due to inadequate assessment of neuronal loss. Indeed, viral vectors usually cause partial loss of nigrostriatal dopaminergic neurons (Kirik et al. 2002). Because the copy number per cell cannot be assessed, it is possible that differential expression levels account for the failure of all dopaminergic neurons to die. Although this reproduces more faithfully intermediate stages of PD in human than the massive lesions usually induced by toxins, the assessment of the lesions requires sophisticated stereological techniques, sensitive immunohistochemical assessment of terminal loss in the striatum, and more specialized behavioral tests than routinely used to evaluate large dopaminergic cell loss in toxin-based models. This may explain why little information is available so far on mechanisms of neuroprotection in this model.

One intriguing observation has been the lack of protection afforded by GDNF in viral-based alpha-synuclein models (Lo Bianco et al. 2004a). This contrasts with the ability of this neurotrophic factor to effectively protect nigrostriatal dopaminergic neurons from toxin-induced cell death (Bensadoun et al. 2000; Zheng et al. 2005) The negative finding is particularly interesting in view of the mixed results of GDNF trials in PD patients to date. Time will tell if this observation underscores a lack of predictive value of the toxin models or if the insult caused by gene delivery of alpha-synuclein is simply too massive to respond to neuroprotective strategy. Interestingly recent results suggest that this is not the case. Indeed, overexpression of parkin, an E3 ligase that is mutated in numerous cases of early onset, recessive parkinsonism, protects against alpha-synuclein toxicity in these models (Lo Bianco et al. 2004b; Yamada et al. 2005). Although beyond the scope of this review, a distinct advantage of gene transfer is that it can be used in primates, thus producing a badly needed primate model of synucleopathy (Eslamboli et al. 2007).

Where do we go from here?

Its complexity will likely continue to restrict the use of alpha-synuclein induced overexpression by gene transfer for testing neuroprotective drugs. However, this model is particularly useful to test specific pathological hypotheses. The well controlled regional and temporal over-expression and the lack of expression during embryonic and post-natal development, which may better mimic disease conditions and avoid the upregulation of defense mechanisms are

distinct advantages. For example, this approach is ideal to determine differences in regional vulnerability of neurons to the transgene (Maingay et al. 2006), and whether different forms of alpha-synuclein (mutated, truncated, constitutively phosphorylated for example) are more pathogenic than others in vivo. However, it will not help resolve the role of multiple affected neuronal systems in the development of the disease since only a subset of the neurons are transduced in these models. Here again, the limitations of the model should not overshadow its value. With appropriate expertise and controls, over-expression of alpha-synuclein by gene transfer is a valuable addition to our arsenal of useful PD models.

In conclusion, the wealth of alpha-synuclein over-expression models in rodents is a clear indication that not a single model can fulfill all experimental needs. One danger is that the shortcomings of available models may discourage their use and delay progress in a field that is ripe for major therapeutic advances. This brief review highlights some of the considerations that should guide the choice of the existing models for immediate studies and some directions for future work. The discovery of the first PD-causing mutation in alpha-synuclein 10 years ago (Polymeropoulos et al. 1997) already had a major impact on our understanding of PD. It is time to take full advantage of even the imperfect models that evolved from this discovery.

References

Bensadoun JC, Deglon N, Tseng JL, Ridet JL, Zurn AD, Aebischer P. Lentiviral vectors as a gene delivery system in the mouse midbrain: cellular and behavioral improvements in a 6-OHDA model of Parkinson's disease using GDNF. Exp Neurol 2000;164:15–24. [PubMed: 10877911]

Bove J, Prou D, Perier C, Przedborski S. Toxin-induced models of Parkinson's disease. NeuroRx 2005;2:484–494. [PubMed: 16389312]

Chen L, Feany MB. Alpha-synuclein phosphorylation controls neurotoxicity and inclusion formation in a Drosophila model of Parkinson disease. Nat Neurosci 2005;8:657–663. [PubMed: 15834418]

Dauer W, Przedborski S. Parkinson's disease: mechanisms and models. Neuron 2003;39:889–909. [PubMed: 12971891]

Eslamboli A, Romero-Ramos M, Burger C, Bjorklund T, Muzyczka N, Mandel RJ, Baker H, Ridley RM, Kirik D. Long-term consequences of human alpha-synuclein overexpression in the primate ventral midbrain. Brain 2007;130:799–815. [PubMed: 17303591]

Fernagut PO, Chesselet MF. Alpha-synuclein and transgenic mouse models. Neurobiol Dis 2004;17:123–130. [PubMed: 15474350]

Fernagut P-O, Hutson CB, Fleming SM, Tetreault N, Salcedo J, Masliah E, Chesselet MF. Behavioral and histopathological consequences of paraquat intoxication in mice: effects of alpha-synuclein overexpression. Synapse. 2007In Press

Fleming SM, Chesselet MF. Behavioral phenotypes and pharmacology in genetic mouse models of Parkinsonism. Behav Pharmacol 2006;17:383–391. [PubMed: 16940759]

Fleming SM, Fernagut PO, Chesselet MF. Genetic mouse models of parkinsonism: strengths and limitations. NeuroRx 2005;2:495–503. [PubMed: 16389313]

Fleming SM, Salcedo J, Fernagut PO, Rockenstein E, Masliah E, Levine MS, Chesselet MF. Early and progressive sensorimotor anomalies in mice overexpressing wild-type human alpha-synuclein. J Neurosci 2004;24:9434–9440. [PubMed: 15496679]

Fleming SM, Salcedo J, Hutson CB, Rockenstein E, Masliah E, Levine MS, Chesselet MF. Behavioral effects of dopaminergic agonists in transgenic mice overexpressing human wildtype alpha-synuclein. Neuroscience 2006a;142:1245–1253. [PubMed: 16934409]

Fleming SM, Tetreault NA, Masliah E, Chesselet M. Alterations in olfactory function in transgenic mice overexpressing human wildtype alpha synuclein. Neurosci. 2006bAbst. 75.9

Follmer C, Romao L, Einsiedler CM, Porto TC, Lara FA, Moncores M, Weissmuller G, Lashuel HA, Lansbury P, Neto VM, Silva JL, Foguel D. Dopamine affects the stability, hydration, and packing of protofibrils and fibrils of the wild type and variants of alpha-synuclein. Biochemistry 2007;46:472–482. [PubMed: 17209557]

Freichel C, Neumann M, Ballard T, Muller V, Woolley M, Ozmen L, Borroni E, Kretzschmar HA, Haass C, Spooren W, Kahle PJ. Age-dependent cognitive decline and amygdala pathology in alpha-synuclein transgenic mice. Neurobiol Aging 2007;28:1421–1435. [PubMed: 16872721]

Gispert S, Del Turco D, Garrett L, Chen A, Bernard DJ, Hamm-Clement J, Korf HW, Deller T, Braak H, Auburger G, Nussbaum RL. Transgenic mice expressing mutant A53T human alpha-synuclein show neuronal dysfunction in the absence of aggregate formation. Mol Cell Neurosci 2003;24:419–429. [PubMed: 14572463]

Halliday GM, Del Tredici K, Braak H. Critical appraisal of brain pathology staging related to presymptomatic and symptomatic cases of sporadic Parkinson's disease. J Neural Transm Suppl 2006:99–103. [PubMed: 17017515]

Hashimoto M, Rockenstein E, Mante M, Crews L, Bar-On P, Gage FH, Marr R, Masliah E. An antiaggregation gene therapy strategy for Lewy body disease utilizing beta-synuclein lentivirus in a transgenic model. Gene Ther 2004;11:1713–1723. [PubMed: 15483670]

Hashimoto M, Rockenstein E, Mante M, Mallory M, Masliah E. beta-Synuclein inhibits alpha-synuclein aggregation: a possible role as an anti-parkinsonian factor. Neuron 2001;32:213–223. [PubMed: 11683992]

Jackson-Lewis V, Przedborski S. Protocol for the MPTP mouse model of Parkinson's disease. Nat Protoc 2007;2:141–151. [PubMed: 17401348]

Kirik D, Bjorklund A. Modeling CNS neurodegeneration by overexpression of disease-causing proteins using viral vectors. Trends Neurosci 2003;26:386–392. [PubMed: 12850435]

Kirik D, Rosenblad C, Burger C, Lundberg C, Johansen TE, Muzyczka N, Mandel RJ, Bjorklund, A. Parkinson-like neurodegeneration induced by targeted overexpression of alpha-synuclein in the nigrostriatal system. J Neurosci 2002;22:2780–2791. [PubMed: 11923443]

Klein RL, King MA, Hamby ME, Meyer EM. Dopaminergic cell loss induced by human A30P alpha-synuclein gene transfer to the rat substantia nigra. Hum Gene Ther 2002;13:605–612. [PubMed: 11916484]

Klucken J, Shin Y, Masliah E, Hyman BT, McLean PJ. Hsp70 Reduces alpha-Synuclein Aggregation and Toxicity. J Biol Chem 2004;279:25497–25502. [PubMed: 15044495]

Langston JW, Ballard P, Tetrud JW, Irwin I. Chronic Parkinsonism in humans due to a product of meperidine-analog synthesis. Science 1983;219:979–980. [PubMed: 6823561]

Lauwers E, Debyser Z, Van Dorpe J, De Strooper B, Nuttin B, Baekelandt V. Neuropathology and neurodegeneration in rodent brain induced by lentiviral vector-mediated overexpression of alpha-synuclein. Brain Pathol 2003;13:364–372. [PubMed: 12946025]

Lee VM, Trojanowski JQ. Mechanisms of Parkinson's disease linked to pathological alpha-synuclein: new targets for drug discovery. Neuron 2006;52:33–38. [PubMed: 17015225]

Levine MS, Cepeda C, Hickey MA, Fleming SM, Chesselet MF. Genetic mouse models of Huntington's and Parkinson's diseases: illuminating but imperfect. Trends Neurosci 2004;27:691–697. [PubMed: 15474170]

Li AA, Mink PJ, McIntosh LJ, Teta MJ, Finley B. Evaluation of epidemiologic and animal data associating pesticides with Parkinson's disease. J Occup Environ Med 2005;47:1059–1087. [PubMed: 16217247]

Li W, West N, Colla E, Pletnikova O, Troncoso JC, Marsh L, Dawson TM, Jakala P, Hartmann T, Price DL, Lee MK. Aggregation promoting C-terminal truncation of alpha-synuclein is a normal cellular process and is enhanced by the familial Parkinson's disease-linked mutations. Proc Natl Acad Sci U S A 2005;102:2162–2167. [PubMed: 15684072]

Litvan I, Chesselet MF, Gasser T, Di Monte DA, Parker D Jr, Hagg T, Hardy J, Jenner P, Myers RH, Price D, Hallett M, Langston WJ, Lang AE, Halliday G, Rocca W, Duyckaerts C, Dickson DW, Ben-Shlomo Y, Goetz CG, Melamed E. The etiopathogenesis of Parkinson disease and suggestions for future research. Part II. J Neuropathol Exp Neurol 2007;66:329–336. [PubMed: 17483689]

Lo Bianco C, Deglon N, Pralong W, Aebischer P. Lentiviral nigral delivery of GDNF does not prevent neurodegeneration in a genetic rat model of Parkinson's disease. Neurobiol Dis 2004a;17:283–289. [PubMed: 15474365]

Lo Bianco C, Ridet JL, Schneider BL, Deglon N, Aebischer P. alpha - Synucleinopathy and selective dopaminergic neuron loss in a rat lentiviral-based model of Parkinson's disease. Proc Natl Acad Sci U S A 2002;99:10813–10818. [PubMed: 12122208]

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 2004b;101:17510–17515. [PubMed: 15576511]

Maingay M, Romero-Ramos M, Carta M, Kirik D. Ventral tegmental area dopamine neurons are resistant to human mutant alpha-synuclein overexpression. Neurobiol Dis 2006;23:522–532. [PubMed: 16806952]

Masliah E, Rockenstein E, Adame A, Alford M, Crews L, Hashimoto M, Seubert P, Lee M, Goldstein J, Chilcote T, Games D, Schenk D. Effects of alpha-synuclein immunization in a mouse model of Parkinson's disease. Neuron 2005;46:857–868. [PubMed: 15953415]

Masliah E, Rockenstein E, Veinbergs I, Sagara Y, Mallory M, Hashimoto M, Mucke L. beta-amyloid peptides enhance alpha-synuclein accumulation and neuronal deficits in a transgenic mouse model linking Alzheimer's disease and Parkinson's disease. Proc Natl Acad Sci U S A 2001;98:12245–12250. [PubMed: 11572944]

McLin JP, Thompson LM, Steward O. Differential susceptibility to striatal neurodegeneration induced by quinolinic acid and kainate in inbred, outbred and hybrid mouse strains. Eur J Neurosci 2006;24:3134–3140. [PubMed: 17156374]

Melrose HL, Lincoln SJ, Tyndall GM, Farrer MJ. Parkinson's disease: a rethink of rodent models. Exp Brain Res 2006;173:196–204. [PubMed: 16639500]

Miller RM, Kiser GL, Kaysser-Kranich T, Casaceli C, Colla E, Lee MK, Palaniappan C, Federoff HJ. Wild-type and mutant alpha-synuclein induce a multi-component gene expression profile consistent with shared pathophysiology in different transgenic mouse models of PD. Exp Neurol 2007;204:421–432. [PubMed: 17254569]

Mochizuki H, Yamada M, Mizuno Y. Alpha-synuclein overexpression model. J Neural Transm Suppl 2006:281–284. [PubMed: 17017543]

Mortazavi F, Meurers B, Oh MS, Elashoff D, Chesselet MF. Transcriptome Analysis of Laser-Captured Dopaminergic Neurons in Mice Overexpressing Human Wildtype Alpha-Synuclein. Neurosci. 2007Abst. In press

Neumann M, Muller V, Kretzschmar HA, Haass C, Kahle PJ. Regional distribution of proteinase K-resistant alpha-synuclein correlates with Lewy body disease stage. J Neuropathol Exp Neurol 2004;63:1225–1235. [PubMed: 15624759]

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 1997;276:2045–2047. [PubMed: 9197268]

Rockenstein E, Mallory M, Hashimoto M, Song D, Shults CW, Lang I, Masliah E. Differential neuropathological alterations in transgenic mice expressing alpha-synuclein from the platelet-derived growth factor and Thy-1 promoters. J Neurosci Res 2002;68:568–578. [PubMed: 12111846]

Song DD, Shults CW, Sisk A, Rockenstein E, Masliah E. Enhanced substantia nigra mitochondrial pathology in human alpha-synuclein transgenic mice after treatment with MPTP. Exp Neurol 2004;186:158–172. [PubMed: 15026254]

Springer W, Kahle PJ. Mechanisms and models of alpha-synuclein-related neurodegeneration. Curr Neurol Neurosci Rep 2006;6:432–436. [PubMed: 16928354]

St Martin JL, Klucken J, Outeiro TF, Nguyen P, Keller-McGandy C, Cantuti-Castelvetri I, Grammatopoulos TN, Standaert DG, Hyman BT, McLean PJ. Dopaminergic neuron loss and up-regulation of chaperone protein mRNA induced by targeted over-expression of alpha-synuclein in mouse substantia nigra. J Neurochem 2007;100:1449–1457. [PubMed: 17241127]

Thiruchelvam MJ, Powers JM, Cory-Slechta DA, Richfield EK. Risk factors for dopaminergic neuron loss in human alpha-synuclein transgenic mice. Eur J Neurosci 2004;19:845–854. [PubMed: 15009131]

Tofaris GK, Garcia Reitbock P, Humby T, Lambourne SL, O'Connell M, Ghetti B, Gossage H, Emson PC, Wilkinson LS, Goedert M, Spillantini MG. Pathological changes in dopaminergic nerve cells of the substantia nigra and olfactory bulb in mice transgenic for truncated human alpha-synuclein(1–120): implications for Lewy body disorders. J Neurosci 2006;26:3942–3950. [PubMed: 16611810]

van der Putten H, Wiederhold KH, Probst A, Barbieri S, Mistl C, Danner S, Kauffmann S, Hofele K, Spooren WP, Ruegg MA, Lin S, Caroni P, Sommer B, Tolnay M, Bilbe G. Neuropathology in mice expressing human alpha-synuclein. J Neurosci 2000;20:6021–6029. [PubMed: 10934251]

Volles MJ, Lansbury PT Jr. Zeroing in on the pathogenic form of alpha-synuclein and its mechanism of neurotoxicity in Parkinson's disease. Biochemistry 2003;42:7871–7878. [PubMed: 12834338]

Wakamatsu M, Ishii A, Iwata S, Sakagami J, Ukai Y, Ono M, Kanbe D, Muramatsu SI, Kobayashi K, Iwatsubo T, Yoshimoto M. Selective loss of nigral dopamine neurons induced by overexpression of truncated human alpha-synuclein in mice. Neurobiol Aging. 2006Ahead of pub.

Wakamatsu M, Ishii A, Ukai Y, Sakagami J, Iwata S, Ono M, Matsumoto K, Nakamura A, Tada N, Kobayashi K, Iwatsubo T, Yoshimoto M. Accumulation of phosphorylated alpha-synuclein in dopaminergic neurons of transgenic mice that express human alpha-synuclein. J Neurosci Res 2007;85:1819–1825. [PubMed: 17465029]

Wang, L.; Fleming, SM.; Mulugeta, M.; Fernagut, P-O.; Chesselet, M-F.; Taché, Y. Abnormal Colonic Motility in Mice Overexpressing Human Wildtype Alpha-Synuclein: An Animal Model of Parkinson’s Disease; Amer. Gastroenterology Mtg. Abst; 2005.

Wu N, Cepeda C, Masliah E, Levine MS. Abnormal glutamate and dopamine receptor function in the striatum of alpha-synuclein overexpressing mice. Neurosci. 2005Abst. 85.12

Yamada M, Mizuno Y, Mochizuki H. Parkin gene therapy for alpha-synucleinopathy: a rat model of Parkinson's disease. Hum Gene Ther 2005;16:262–270. [PubMed: 15761265]

Zheng JS, Tang LL, Zheng SS, Zhan RY, Zhou YQ, Goudreau J, Kaufman D, Chen AF. Delayed gene therapy of glial cell line-derived neurotrophic factor is efficacious in a rat model of Parkinson's disease. Brain Res Mol Brain Res 2005;134:155–161. [PubMed: 15790539]

Ziemssen T, Reichmann H. Non-motor dysfunction in Parkinson's disease. Parkinsonism Relat Disord. 2007 Mar 7;[Epub ahead of print]