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14. ABSTRACT Purpose: Alternative splicing is responsible for producing several products from a single transcript and can cause pathogenic changes in RNA in neurodegenerative disease. This proposal tests the hypothesis that regulation of normal splicing is disrupted in Parkinson's disease (PD). Scope: Experiments determined splicing products in the brain and blood of experimental MPTP models of PD. The overall goal was to use splice variants as biomarkers to identify individuals at risk for PD. To date, we have identified and quantified alternatively spliced transcripts for several candidate genes in MPTP models of PD. We also had IRB permission (for only 9 months) to study splicing factors in the blood of PD patients. Major Findings: Mice treated acutely and chronically with MPTP show a shift in the ratio of FosB, RGS9, AChE and Ania6 splice variants in the striatum and blood. Gene expression (in situ hybridization) studies have demonstrated that AChE variant R is upregulated in the striatum and blood after MPTP. No patients were enrolled for the human blood study and that study is closed. Overall we have published four articles and have three in preparation.					
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Introduction

Alternative splice products of different genes have been identified in the brains of animals models of experimental parkinsonism and confirmed in Parkinson's disease (PD) patients (Tekumalla et al., 2001). Such variants are produced in different proportions in healthy individuals, which means that alternative splice variants could be useful biomarkers of the disease state. During this past year we have identified several changes in the ratios of splice isoforms of mRNA transcripts in the brain and blood of mice treated chronically or acutely with MPTP. We have published or submitted for publication some of these findings and in addition, are carrying out gene expression studies to localize the aberrant splice products.

Body

We have made good progress during the final year of this grant on the mouse work but were disappointed that we were unable to recruit patients for the human blood study. We outline our key accomplishments under each Objective of the original Statement of Work.

Objective 1: To identify abnormal splice variants of genes involved in the development and progression of Parkinsonism in the brain and blood of chronic rodent models of PD.

We have completed all parts of Objective 1 in the Statement of Work. The new data obtained over the past year includes splice variants that were quantified and normalized to 18S rRNA. By quantifying in this manner we are able to examine the steady state levels of RNA, taking into account changes that occur due to transcription and RNA stability, in addition to those that occur because of a dysregulation of splicing. We now express the data as splice variant1/ splice variant2, splice variant1/18S rRNA and splice variant2/18S rRNA. We summarize the sequences that we tested in table 1.

Table 1. sequences tested on mouse brain and blood

PCR	
Primer	Sequence
Ache-R: Forward Primer	5' CCCCATGACCCTCGAGACT 3'
Ache-R: Reverse Primer	5' CCTCCTTCCAACCCTTGCC 3'
Ache-S: Forward Primer	5' TCTTTGAACACCGTGCCTCC 3'
Ache-S: Reverse Primer	5' CTCCGCCTCGTCCAGAGTAT 3'
Ania6/6a: Reverse Primer	5' GAA AGC GAA CAA AGA CAT TGG TT 3'
Ania6: Forward Primer	5' TCA AGG CAG AGA GGA GGG TG 3'
Ania6a: Forward Primer	5' TGC TGT GGG GAA GTG GTT AG 3'
CD40 (Var. 1): Forward Primer	5' GCT CAG CAC ACG CCC TGT A 3'
CD40 (Var. 1): Reverse Primer	5' ATA GAG AAA CAC CCC GAA AAT GG 3'
CD40 (Var. 2): Forward Primer	5' GCT ATG GGG CTG CTT GTT GAC AG 3'
CD40 (Var. 2): Forward Primer	5' GCC AGG GAT ACA GGG CGT GTG 3'
DJ1: Forward Primer	5' AAT GAT TTG TCC AGA TAC CAG TC 3'
DJ1: Reverse Primer	5' TTT TCT TTT TCT CTC TCC CTT CT 3'
Drd2: Forward Primer	5' ATT GTC TGG GTC CTG TCC TTC A 3'
Drd2: Reverse Primer	5' TCT GGT TTG GCA GGA CTG TCA G 3'
FAIM: Forward Primer	5' GAA GGC AGT AGG ATG CTG GG 3'
FAIM: Reverse Primer	5' TAC AAA CTC GCC CGC TGT CT 3'
FGFR1 IIIb: Reverse Primer	5' TAC ACA CAT ACT CCC CGC TCT 3'
FGFR1 IIIc: Reverse Primer	5' CTT CCA GAA CGG TCA ACC AT 3'
FGFR1: Forward Primer	5' TGC CTG CCA ACA AGA CAG T 3'
FosB: Forward primer	5' AAAAGGCAGAGCTGGAGTCG 3'
FosB: Reverse primer	5' GTACGAAGGGCTAACAACGG 3'
Gabrg2: Forward Primer	5' TCT CTG CCC AAG GTC TCC T 3'
Gabrg2: Reverse Primer	5' TGC CAT CCA AAC ACT CAT AG 3'
GNAS: Forward Primer	5' GCA GCG TGA GGC CAA CAA AAA GAT 3'
GNAS: Reverse Primer	5' CCA CTC TGA ACT GGT TCT CGG GGT 3'
Gria2 (Flip): Forward Primer	5' TCT CCT CCT ACA CGG CTA ACT 3'
Gria2 (Flip): Reverse Primer	5' GCA AGA TTT ACT GGG GTT CT 3'
Gria2 (Flop): Forward Primer	5' CTC CTC CTA CAC GGC TAA CTT A 3'
Gria2 (Flop): Reverse Primer	5' CCG CAC TCT CCT TTG TCG TA 3'
Grin1: Forward Primer	5' ATG TGA CTC CCG CAG CAA TGC 3'
Grin1: Reverse Primer	5' ACC AGG AAG GCT GCC AGG TTG 3'
Homer1 Long: Forward Primer	5' GCA TTG CCA TTT CCA CAT AG 3'
Homer1 Long: Reverse Primer	5' TGC CCC TCC AGG TCT TTA T 3'
Homer1 Short: Forward Primer	5' ATA AAT AGC ACC ATC ACA CCA AA 3'
Homer1 Short: Reverse Primer	5' CTG AAA CCC AAA TGA CTT CCA 3'
NDUFS4: Forward Primer	5' TGG GGC GAA GGG CAA TGG 3'
NDUFS4: Reverse Primer	5' TGG AGA GGG GGT CAG CGG T 3'
OXR1: Forward Primer	5' GAC CAC TTG TAT GCC TTC TTC AT 3'
OXR1: Reverse Primer	5' TTG AGT TGA TGT CTT CCC TTG T 3'
PALM: Forward Primer	5' GAG CAA AAG TCA GAA ACC TTG GTG 3'
PALM: Reverse Primer	5' GCC TTG TGA ATG AGT TCG TCC A 3'
PSM4: Forward Primer	5' GGA TGA GAT TCC AGC ACT GTC CG 3'

PSM4: Reverse Primer	5' ACC GAG GCG TTG GGC TTG AG 3'
RGS9 2: Forward primer	5' GGCAGCTGGAAGAAGAAGAGAA 3'
RGS9-1: Forward primer	5' GATTCTTACGCACGCTATTTGA 3'

Objective 2: To determine if the distribution of those splice variants found in rodent models of Parkinsonism correlate with regions in the brain that are affected in PD.

Quantitative *in situ* hybridization (ISH) has been carried out on the striatum and midbrains of mice treated chronically MPTP. In this work, we have completed studies of the splice variants of AChE and ANIA-6 (cyclin L1). The data from these studies are being prepared for publication.

Objective 3: To determine if the splice variants whose regulation is altered in rodent models are altered in the blood of PD patients compared to age-matched controls.

We had IRB approval from the USAMRMC and from the two Universities with whom we collaborate for less than a year (9 months only). No patients were enrolled during that time and therefore this study closed prematurely. We are presently applying for further funding from private foundations and the NIH to set up a larger study of patient blood.

Key Research Accomplishments:

- Successfully extracted RNA from blood, brain (striatum and substantia nigra) and nasal epithelium of mice treated with MPTP, and quantified splice variants using TaqMan assays (Perkin-Elmer-Applied Biosystems)
- Successfully correlated changes in splice variant ratios with the loss of dopaminergic neurons from the substantia nigra, poor performance on behavioral tests and dopamine levels in the striatum.
- Gene Expression studies showed an increase in AChE-S gene expression in the striatum and a decrease in AChE-R mRNA in the substantia nigra after chronic MPTP treatment.

Reportable Outcomes

Published or submitted for publication:

- Potashkin JA, Meredith GE (2006) The role of oxidative stress in the dysregulation of gene expression and protein metabolism in neurodegenerative disease. *Antioxid Redox Signal*, 8:144-151.
- Potashkin JA, Kang UJ, Loomis PA, Jodelka FM, Ding Y, Meredith GE (2007) MPTP administration in mice changes the ratio of splice isoforms of fosB and rgs9. *Brain Res* 1182:1-10.
- Potashkin JA, Kang UJ, Loomis PA, Ding Y, Jodelka FM, Meredith GE (2007) Dysregulation of AChE splicing in acute and chronic models of Parkinson's disease. Eukaryotic RNA processing, Cold Spring Harbor, NY.
- Meredith GE, Totterdell S, Potashkin JA, Surmeier DJ (2008) Modeling PD pathogenesis in mice: Advantages of a chronic MPTP model. *Parkinsonism Relat Disord* 14: S112-S115.
- Meredith GE, Sonsalla PK, Chesselet M-F (2008) Animal models of Parkinson's disease progression. *Acta Neuropathol* 115:385-398.
- Potashkin JA, Loomis PA, Jodelka FM, Meredith GE (2008) Cyclin L1 splicing is dysregulated after MPTP treatment to mice. Submitted.
- Potashkin JA, Loomis PA, Jodelka FM, Meredith GE (2008) MPTP exposure alters expression of Ndufs4 variants in the mouse brain Submitted
- Potashkin JA, Loomis PA, Jodelka FM, Meredith GE (2008) Dysregulation of AChE splicing in acute and chronic MPTP mouse models of Parkinson's disease. In preparation.

Conclusion

The final year of this grant has seen the completion of splicing and gene expression studies in the brain and blood of the MPTP mouse model. We were unable to enroll patients for the human study during the past 9 months. However, we hope to obtain new funding to study the blood of newly diagnosed human PD patients in order to establish the transcripts that we identified in the mouse model as biomarkers of disease.

References

Tekumalla PK, Calon F, Rahman Z, Birdi S, Rajput AH, Hornykiewicz O, Di Paolo T, Bedard PJ, Nestler EJ (2001) Elevated levels of DeltaFosB and RGS9 in striatum in Parkinson's disease. *Biol Psychiatry* 50:813-816.

Appendix I. Meredith GE, Totterdell S, Potashkin JA, Surmeier DJ (2008) Modeling PD pathogenesis in mice: Advantages of a chronic MPTP model. *Parkinsonism Relat Disord* 14: S112-S115.

Appendix II. Meredith GE, Sonsalla PK, Chesselet M-F (2008) Animal models of Parkinson's disease progression. *Acta Neuropathol* 115:385-398.

REVIEW (published in Parkinsonism and Related Disorders)

Modeling PD pathogenesis in mice: advantages of a chronic MPTP protocol

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Key words: probenecid, L-type calcium channel, grid test, alpha-synuclein, substantia nigra, dopamine, isradipine

Short title: Chronic MPTP/p model

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Abstract

Formidable challenges for Parkinson's disease (PD) research are to understand the processes underlying nigrostriatal degeneration and how to protect the dopamine neurons. Fundamental research relies on good animal models that demonstrate the pathological hallmarks and motor deficits of PD. Using a chronic regimen of 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine and probenecid (MPTP/p) in mice, dopamine cell loss exceeds 60%, extracellular glutamate is elevated, cytoplasmic inclusions are formed and inflammation is chronic. Nevertheless, isradipine, an L-type calcium-channel blocker, attenuates the degeneration. These data support the validity of the MPTP/p model for unravelling the degenerative processes in PD and testing therapies that slow their progress.

1. Introduction

Parkinson's disease (PD) is characterized by progressive loss of dopamine neurons and terminals from the nigrostriatal pathway and by a slow onset of motor symptoms. To provide insight into the pathophysiological processes of this disease, animal models should mimic as many of the clinical features as possible. The loss of the dopaminergic pathway can be replicated in rodents using various surgical, toxic or genetic approaches. Over the past couple of decades, one neurotoxin, 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP), has become a widely used method for modeling PD. However, for most MPTP models, the loss of dopamine is rapid and not progressive, and the motor disability is often difficult to demonstrate, especially when tested some time after toxin application [1]. A model that shows great promise, particularly in its progressive nature, involves the administration of MPTP and an adjuvant, probenecid (MPTP/p), that blocks the rapid clearance of the toxin and its metabolites [2]. Chronic MPTP/p treatment produces many of the pathological hallmarks and motor deficits of PD, making it an excellent choice for studies of pathogenesis, for testing neuroprotective therapies and developing biomarkers to detect the disease presymptomatically [3, 4]. This review covers the key features of this model and discusses its applicability to neuroprotective strategies.

2. Preparation of the chronic MPTP/p model

Male C57/bl mice, initially weighing 20–24g, are injected with 10 doses of MPTP hydrochloride (25mg/kg in saline, s.c.) and probenecid (250mg/kg in tris-HCl buffer) over 5 weeks at 3.5-day intervals. Control mice are injected with vehicle (saline or probenecid) in the same volume and on the same schedule. Three days before treatment, and each week thereafter, mice are tested for coordination and rigidity using the grid test [1, 3, 5]. Briefly, mice are placed in the center of a wire mesh grid, which is then rotated 180 degrees to suspend them upside down. Mice are allowed to move freely on the grid and their movements are filmed for 60 s. Forepaw foot-faults and total forepaw steps are recorded for each of three trials and a ratio (foot-faults/total steps) is established per mouse. Data are pooled for each group and paired Student's *t*-tests compare within-groups' grid activity pre- and post-treatments, and unpaired *t*-tests compare between-groups' grid performance.

After the final behavioral test, mice are perfused transcardially with fixative and their brains prepared for light (LM) or electron (EM) microscopy. For LM, adjacent sections are immunoreacted for tyrosine hydroxylase (TH), Mac-1 and alpha-synuclein. The total number of

TH-immunoreactive, Nissl-stained neurons and Mac-1-immunoreactive cells (microglia) are estimated with optical disectors (optical fractionator approach) using dedicated software (Stereoinvestigator, Microbrightfield, Williston, VT). Inclusions are identified using LM, confocal microscopy and EM. Sections for EM are immunoreacted for TH and prepared for examination in a Philips 400 electron microscope. Mice were killed by cervical dislocation and decapitation and then striatal dopamine concentration is determined electrochemically (Coulochem II, ESA, Chelmsford, MA).

3. Dopamine loss, motor dysfunction, inflammation and inclusion formation

The chronic MPTP/p model shows a significant reduction in the number of neurons in the substantia nigra pars compacta (SNpc). Shortly after MPTP/p treatment, approximately 50% of dopamine neurons are lost, increasing to nearly 70% 3 weeks after toxin treatment (Table 1). Striatal dopamine levels are reduced by 90–93% within a week, and by 70–80% of the total at 3 to 24 weeks after MPTP/p treatment [4]. The low level of striatal dopamine is matched by a significant loss of TH-immunopositive fibers throughout the caudate putamen, especially in central and medial parts [2]. Dopamine loss correlates well with motor deficits. As early as 3 days post-MPTP/p treatment, mice show a significant disability on the grid and the impaired performance persists up to 6 months post-MPTP/p treatment. Typically, vehicle-treated mice perform significantly better (ratio of foot-faults/total steps: 0.036 ± 0.01) than MPTP/p-treated animals (0.167 ± 0.04 ; $p < 0.001$, Student's *t*-test).

There is a strong inflammatory response in the SN 3 weeks after MPTP/p treatment (density of microglia for the MPTP/p group: $2.76 \pm 0.04 \times 10^4/\text{mm}^2$ versus vehicle [probenecid] group: $2.39 \pm 0.09 \times 10^4/\text{mm}^2$; $p < 0.05$, Student's *t*-test). Reactive microglia with large cell bodies and short processes are also found after MPTP/p treatment and persist for months [6].

The formation of inclusion bodies has been demonstrated for several chronic MPTP models, but not for acute or subchronic models [7-11]. Presumably, the slow administration of MPTP/p can induce prolonged damage to mitochondria and precipitate alpha-synuclein toxicity, resulting in cytoplasmic accumulation of alpha-synuclein and ubiquitin proteins. For the MPTP/p model, inclusions have been identified in cell bodies and dendrites of TH-immunoreactive neurons as early as 2 to 3 weeks after toxin administration. These inclusions immunostain for alpha-synuclein, DJ-1 and ubiquitin (Fig. 1 [7, 8]) and, at the EM level, are granular, contain lipid

droplets, proteinaceous deposits and parallel membranes (Fig. 2 [7]). Ultrastructurally, the granules have the appearance of lipofuscins or secondary lysosomes (Fig. 2), cellular organelles that accumulate with age but at a significantly faster rate in neurodegenerative disease [12]. In PD, lipofuscins are closely associated with lipid droplets and neuromelanin, and may be important for the development of Lewy bodies [12].

4. Cell death in the MPTP/p model

MPTP intoxication rapidly and persistently depletes ATP and increases reactive oxygen and nitrogen species molecules that induce cell death pathways. In acute or subchronic MPTP models, less than half of the SNpc dopaminergic neurons are destroyed, whereas nigrostriatal degeneration with the chronic MPTP/p regimen is more extensive (Table 1 [2]). This is because striatal dopamine depletion peaks within 24 hours after a single dose of MPTP, but that loss is extended with MPTP/p, presumably due to the probenecid's competitive block of active transport of the toxin at the kidney and blood-brain-barrier [2]. This means that more dopamine neurons die over time. The chronic MPTP/p model also reveals numerous pathological features, such as persistent inflammation, alpha-synuclein-positive inclusions, and aberrant elevations in extracellular glutamate (Meredith and Meshul, unpublished results), all of which would increase vulnerability to calcium (Ca^{2+}) influx and excitotoxicity. Prolonged intervention with compounds that reduce Ca^{2+} -dependent cellular stress could, therefore, be tested with this model.

5. Neuroprotection

Adult SNpc dopamine neurons are Ca^{2+} -dependent autonomous pacemakers, the basal activity of which is driven by the relatively rare, voltage-dependent, L-type Ca^{2+} channel $\text{Ca}_v1.3$ [13]. Pacemaking elevates cytosolic Ca^{2+} , and would therefore harm neurons that are energy-compromised through mitochondrial stress (as in PD [12, 14]). If the Ca^{2+} dependence of pacemaking could be changed, perhaps through the blockade of $\text{Ca}_v1.3$, some protection may be afforded to dopamine neurons. We conducted *in vivo* experiments using mice administered isradipine, a potent L-type Ca^{2+} channel blocker, during treatment with MPTP/p. Mice were implanted with extended release pellets with biodegradable-carrier bound isradipine (60 days, 3 $\mu\text{g/g/day}$) or inert placebo pellets (Tocris, Ellisville, MO) 1 week before toxin or vehicle treatment. One week after treatment, mice were tested on the grid and, 24 hours later, euthanized and TH-immunoreacted so that Nissl-counterstained neurons in the SNpc could be counted.

Isradipine significantly improved performance compared to mice implanted with placebo and, although MPTP/p-treated mice with isradipine lost significantly more SNpc neurons than those treated with vehicle, isradipine attenuated the loss compared to MPTP/p-treated, placebo-implanted mice (Table 1 [3]). Protection was not due to isradipine affecting MPTP metabolism, because brain 1-methyl-4-phenylpyridinium ion (MPP+) levels did not differ between toxin-treated groups [3].

In conclusion, mice treated with MPTP/p exhibit many features of PD, including dopamine cell loss, motor deficits, inclusion formation and inflammation. The model is thus an attractive choice for testing neuroprotective strategies or for developing biomarkers for early detection of disease.

Acknowledgements

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Conflict of interest statement

Gloria E. Meredith None declared.

Susan Totterdell None declared.

Judith A. Potashkin None declared.

D. James Surmeier None declared.

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References

- [1] Meredith GE, Kang UJ. Behavioral models of Parkinson's disease in rodents: a new look at an old problem. *Mov Disord* 2006; 21:1595-606.

- [2] Petroske E, Meredith GE, Callen S, Totterdell S, Lau YS. Mouse model of Parkinsonism: a comparison between subacute MPTP and chronic MPTP/probenecid treatment. *Neuroscience* 2001; 106:589-601.
- [3] Chan CS, Guzman JN, Ilijic E, Mercer JN, Rick C, Tkatch T, et al. 'Rejuvenation' protects neurons in mouse models of Parkinson's disease. *Nature* 2007; 447:1081-6.
- [4] Potashkin JA, Kang UJ, Loomis PA, Jodelka FM, Ding Y, Meredith GE. MPTP administration in mice changes the ratio of splice isoforms of fosB and rgs9. *Brain Res.* 2007; 28:1182:1-10.
- [5] Tillerson JL, Miller, G.W. Grid performance test to measure behavioral impairment in the MPTP-treated-mouse model of parkinsonism. *J Neurosci Meth* 2003; 123:189-200.
- [6] Meredith GE, Dervan AG, Totterdell S. Activated microglial persist in the substantia nigra of a chronic MPTP mouse model of Parkinson's disease. In: Bolam JP, Ingham CA, Magill P, editors. *Basal Ganglia VIII*. New York, NY: Plenum Press, 2005. p 341-8.
- [7] Meredith GE, Totterdell S, Petroske E, Santa Cruz K, Callison RC, Jr., Lau YS. Lysosomal malfunction accompanies alpha-synuclein aggregation in a progressive mouse model of Parkinson's disease. *Brain Res* 2002; 956:156-65.
- [8] Jin J, Meredith GE, Chen L, Zhou Y, Xu J, Shie FS, et al. Quantitative proteomic analysis of mitochondrial proteins: relevance to Lewy body formation and Parkinson's disease. *Mol Brain Res* 2005; 134:119-38.
- [9] Fornai F, Schluter OM, Lenzi P, Gesi M, Ruffoli R, Ferrucci M, et al. Parkinson-like syndrome induced by continuous MPTP infusion: convergent roles of the ubiquitin-proteasome system and alpha-synuclein. *Proc Natl Acad Sci USA* 2005; 102:3413-8.
- [10] Shimoji M, Zhang L, Mandir AS, Dawson VL, Dawson TM. Absence of inclusion body formation in the MPTP mouse model of Parkinson's disease. *Brain Res Mol Brain Res* 2005; 134:103-8.
- [11] Yazdani U, German DC, Liang CL, Manzino L, Sonsalla PK, Zeevalk GD. Rat model of Parkinson's disease: Chronic central delivery of 1-methyl-4-phenylpyridinium (MPP(+)). *Exp Neurol* 2006; 200:172-83.
- [12] Meredith GE, Halliday, G.M., Totterdell, S. A critical review of the development and importance of proteinaceous aggregates in animal models of Parkinson's disease: New insights into Lewy body formation. *Parkinsonism Relat Disord* 2004;10:191-202.
- [13] Mercuri NB, Bonci A, Calabresi P, Stratta F, Stefani A, Bernardi G. Effects of dihydropyridine calcium antagonists on rat midbrain dopaminergic neurones. *Br J Pharmacol* 1994; 113:831-8.
- [14] Beal MF. Mitochondrial dysfunction in neurodegenerative diseases. *Biochim Biophys Acta* 1998;1366:211-23.

Table 1. Stereological results for TH-immunoreactive, Nissl-stained neurons in the SNpc, including reference volume (Vr) and estimated total number of neurons. Data are derived from two experiments, separated below by double lines.

Treatment	N	Vr $\pm$ SEM (mm ³)	Total number of neurons $\pm$ SEM (x 10 ³)	Coefficient of error
MPTP/p	8	0.164 $\pm$ 0.003 [†]	3.139 $\pm$ 0.12 ^a	0.11
Probenecid	6	0.170 $\pm$ 0.004	9.440 $\pm$ 0.11	0.09
Saline	5	0.178 $\pm$ 0.008	9.672 $\pm$ 0.10	0.10
MPTP/p + placebo	4	0.186 $\pm$ 0.02 [†]	3.669 $\pm$ 0.27 ^a	0.10
MPTP/p + isradipine	7	0.185 $\pm$ 0.019	6.789 $\pm$ 0.56 ^{b,c}	0.08
Saline + isradipine	5	0.193 $\pm$ 0.023	9.607 $\pm$ 0.3	0.07

^a $p < 0.01$ (Student's *t*-test), significantly less than in vehicle-treated group(s).

^b $p < 0.01$ (Student's *t*-test), significantly less than in saline/isradipine-treated group.

^c $p < 0.001$ (Student's *t*-test), significantly greater than in MPTP + placebo group.

[†]No significant difference between groups.

Figures

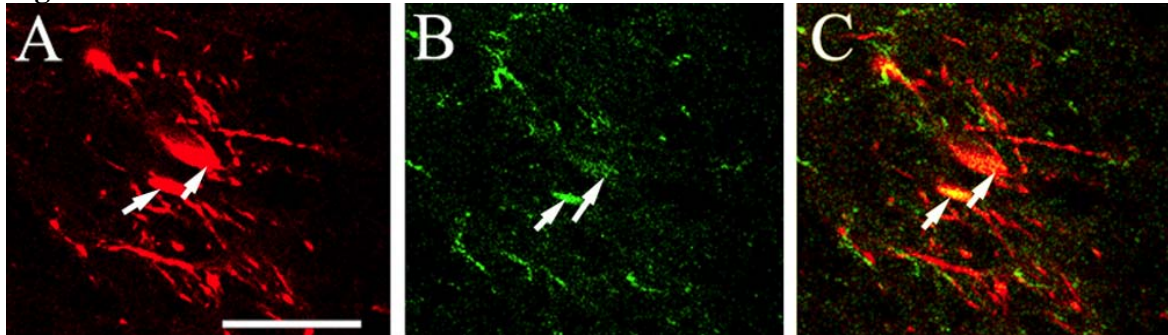


Fig. 1. Images of TH and alpha-synuclein immunoreactivity in the SNpc. (A) TH-immunoreactive neurons, (B) alpha-synuclein-immunoreactive puncta (note the varicosities of alpha-synuclein terminals), and (C) merged image showing the alpha-synuclein-immunoreactive granular inclusions in TH-immunoreactive neurons. Scale bar in A is valid for A, B and C, and equals 25 μ m.

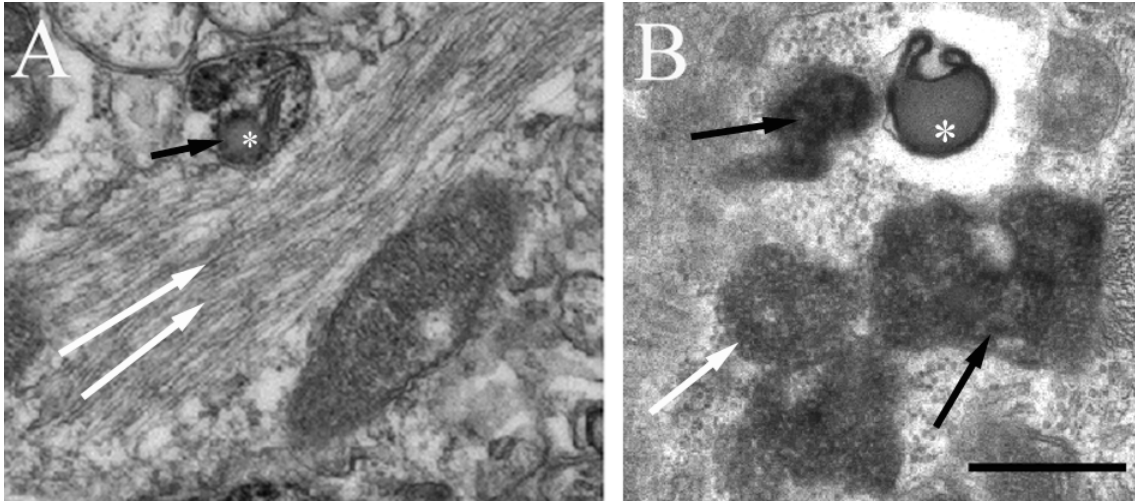


Fig. 2. Ultrastructural appearance of inclusions in the SNpc of MPTP/p-treated mice. (A) A TH-immunoreactive membrane-bound structure is filled with a proteinaceous deposit (black arrows) and an electron lucent lipid deposit (asterisk). Note extracellular parallel fibers (white arrows). (B) Proteinaceous (black arrows) cytoplasmic deposits and lipid (asterisk) in the SNpc. Scale bar in B is valid for A and B, and equals 0.5 μ m.

Animal Models of Parkinson's Disease Progression

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Abstract

Parkinson's disease (PD) is a progressive neurodegenerative disorder whose etiology is not understood. This disease occurs both sporadically and through inheritance of single genes, although the familial types are rare. Over the past decade or so, experimental and clinical data suggest that PD could be a multifactorial, neurodegenerative disease that involves strong interactions between the environment and genetic predisposition. Our understanding of the pathophysiology and motor deficits of the disease relies heavily on fundamental research on animal models and the last few years have seen an explosion of toxin-, inflammation- induced and genetically manipulated models. The insight gained from the use of such models has strongly advanced our understanding of the progression and stages of the disease. The models have also aided the development of novel therapies to improve symptomatic management, and they are critical for the development of neuroprotective strategies. This review critically evaluates these *in vivo* models and the roles they play in mimicking the progression of PD.

Keywords: substantia nigra, MPTP, 6-OHDA, rotenone, LPS, engrail, alpha-synuclein

Introduction

There are many theories on the etiology of Parkinson's disease (PD), but most agree that outside of the rare familial cases, this disorder involves interactions between genetic and environmental factors [64]. The primary neuropathological feature is the profound loss of dopaminergic (DA) nigrostriatal neurons. However, the neuropathology is not restricted to these neurons, for reductions in non-DA cells appear either before or subsequent to the substantia nigra (SN) loss [9, 10, 47]. Other prominent neuropathological features also emerge, including the accumulation of insoluble proteins, such as alpha-synuclein, in cytoplasmic inclusions called Lewy bodies in SN DA neurons and, in some cases, in non-dopaminergic neurons located elsewhere [1, 49].

Investigators rely heavily on rodents to model the features of PD and provide insight into the mechanisms underlying the pathophysiology. However, there is controversy as to which model(s) best represent(s) the progressive nature of PD and whether a model can demonstrate the important distinction between “preclinical” and “clinical” disease states. Among the many models created over recent decades, the most widely used are those that employ toxins, such as 6-hydroxydopamine (6-OHDA), 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP), rotenone, or paraquat, but demonstrating specific and progressive SN cell loss has been disappointing with some protocols. Nevertheless, several models are able to mimic one or more of the stages of PD, particularly if partial or graded lesions are induced.

We have known for decades that neuroinflammation is present in PD. There is an activated microglia response and increased microglial cytokine expression in the SN of PD patients [8, 54]. While the presence of reactive microglia in humans was initially

thought to be a consequence of ongoing neuronal degeneration, we now believe that the microglia contribute to neurodegeneration [87]. Thus, inflammation-based models have been created. Lipopolysaccharide (LPS), an endotoxin derived from the cell wall of gram-negative bacteria, is a potent inducer of inflammation, a powerful activator of microglial cells, and can be used to model neuroinflammation in PD [133]. Progressive features have been demonstrated in some of these models.

Despite recent efforts to develop progressive toxin- or inflammation- based protocols, mouse or rat models created through the expression of genetic mutations may prove to be ideal for modeling disease progression. Indeed, progressive behavioral deterioration, increasing pathology with age, and alterations in motor function that manifest “subclinical” deficits have been demonstrated. The extent to which they reproduce many hallmarks of PD and the mechanisms at work in the sporadic forms of the disease vary greatly. Importantly, a few mouse lines exhibit non-progressive cell loss suggesting they do not reliably reproduce pathophysiological mechanisms of PD. This stresses the need to examine phenotypes at different ages.

In this review, we will discuss the ability of all these models to replicate the progression and extent of DA nigrostriatal loss found in PD and discuss the challenges and caveats of using them as models of preclinical or advanced disease states.

PD Models: Acute or Chronic Delivery of Neurotoxicants

Many different toxins are used to generate DA degeneration. Most are able to potentially inhibit Complex I or enhance the production of reactive oxygen species (ROS) through their effect on mitochondria. Some specifically target the DA neurons through

preferential uptake by transporters. An emphasis of recent research has been on the creation of models where exposure is chronic and damage occurs progressively to mimic human PD. As such, these models can be valuable to define early and late processes associated with neuronal degeneration and evaluate neuroprotective strategies during mid or late stage degeneration, which is when therapy in PD patients is initiated.

6-Hydroxydopamine

The neurotoxin, 6-OHDA, is structurally similar to dopamine and norepinephrine (NE) and has a high affinity for the plasma membrane transporters of these catecholamines [11]. Once inside the neurons, it is readily oxidized and produces hydrogen peroxide and paraquinone, both of which are highly toxic [103]. This toxin does not readily cross the blood-brain-barrier, but when administered directly in the brain, it specifically kills DA and NE neurons and their terminals [58, 61]. Dismethylimipramine injected systemically before 6-OHDA protects NE neurons [11]. The degree of loss of DA neurons and their striatal terminals is dependent upon the location and dose of the toxin, as well as the survival time following the lesion (table 1). However, this toxin does not produce extra-nigral pathology or Lewy body-like inclusions [22, 70].

6-hydroxydopamine is generally administered unilaterally to the SN, medial forebrain bundle (MFB) or striatum. Following delivery of 6-OHDA to the ventral midbrain, most concentrations destroy the SN DA cells within a few hours, and before the striatal terminals disappear [59], but when injected into the MFB, striatal terminals degenerate first, followed by DA cell death (table 1; [138]. Dopaminergic neurons in the ventral tegmental area (VTA) are virtually unaffected, which is similar to DA loss in PD [45]. Some of the earliest work with this toxin introduced it (25-200 μ g) intracisternally, which reduced brain DA levels by 70% and NE by 75% [11]. More recent investigations have used injection concentrations of 4-8 μ g/ μ l of the toxin in the SN or MFB. These latter doses rapidly reduce striatal DA levels by 90% percent and produce a nearly complete destruction of SN neurons and striatal tyrosine hydroxylase (TH)-immunoreactive terminals [45, 122, 138]. Interestingly, Stanic and colleagues [115] found that by 16 weeks after a partial SN lesion (less than 75%), the striatum is completely re-innervated

by TH-immunoreactive fibers and the turning bias demonstrated by amphetamine normalizes (see below), indicating that only large lesions (greater than 75%) can permanently destroy the nigrostriatal pathway.

Among the motor tests used following 6-OHDA lesions, the ‘gold standard’ measures the magnitude of nigrostriatal loss and involves injecting the rat with apomorphine or amphetamine and counting the number of rotations [124]. The rotational tests are complex in that DA uptake inhibitors induce ipsilateral rotation, whereas DA agonists produce contralateral rotation [71]. Systemic injection of levodopa induces a robust contralateral rotation as does the receptor agonist, bromocriptine [99, 124]. Because the 6-OHDA lesion is unilateral, animals show asymmetry in the cylinder and adjusting step motor tests [106]. Therefore, a large 6-OHDA lesion administered to the MFB produces an excellent model of late stage PD and has often been used to screen pharmacotherapies for symptomatic relief. Nevertheless, recent work has created 6-OHDA models of earlier stages of PD, using graded doses of toxin into the MFB (1, 2 or 4 $\mu\text{g}/\mu\text{l}$) and demonstrating abnormal locomotion, balance and posture [122]. Indeed, this model may be more effective in detecting motor abnormalities than other ‘bilateral’ toxin models, because the unilateral nature of the lesion forces a rat to shift its weight abnormally for locomotion and balance, thereby creating quantifiable deficits that are analogous to many seen in PD [60]. Moreover, PD often begins as a unilateral disorder progressing rapidly to bilateral symptoms, and the 6-OHDA model may recapitulate early motor signs, especially with a partial lesion.

When 6-OHDA is injected in the striatum, the loss of DA nigrostriatal pathway is more progressive than with injections in other locations, even though it is dose-dependent [97]. A large toxin dose (20 μg) into the striatum reportedly destroys SN neurons slowly

over weeks reaching a maximum cell loss by 16 weeks post-lesion [105]. Fleming and colleagues [30] gave ascending doses of 6-OHDA to the striatum through a unilateral indwelling cannula over 14 days. They were able to induce a 35% DA cell loss over this period and measure subtle, but significant, behavioral impairments suggesting that this method of delivery produces a progressive, perhaps preclinical, parkinsonism.

MPTP

The identification of MPTP, a synthetic heroin that kills DA neurons, led to it becoming among the most widely used toxins to mimic the hallmarks of PD [38]. This is because the toxic metabolite, MPP⁺ is a potent Complex I inhibitor in DA neurons and postmortem PD brains show Complex I damage [107]. Indeed, MPTP models have been most useful in studies of the molecular changes that underlie mitochondrial dysfunction [22, 24].

In rodents, MPTP is delivered systemically, either i.p. or s.c., and with repeated injections. Rats are not susceptible to the toxin and MPTP potency varies among mouse strains [108]. Despite this, frequent injections and large doses, which are often required to produce significant DA depletion in mice [114], are associated with high mortality and may not produce large scale cell death [48, 95]. This toxin kills DA neurons rapidly at first (table 1), but if injected over time, continues to cause cell death [6].

Three MPTP protocols, with some variation, are widely used. The acute protocol generally involves 4 injections in one day at 2 h intervals. Subacute (or subchronic) administration is a once daily injection over 5-8 days [63, 95]. One chronic regimen utilizes repeated treatments over 5 weeks and requires the co-administration of the

adjuvant probenecid to retard the renal clearance of the toxic metabolites of MPTP [73, 95]. For all these protocols, SN TH immunoreactive neurons disappear rapidly, but this loss may not reflect actual DA cell death if neurons are counted shortly after treatment, since MPTP down-regulates TH gene expression [134]. In the past few years, the introduction of unbiased stereology to count the TH-immunoreactive and the Nissl-stained neurons at least 7 days following any MPTP treatment has provided better estimates of DA cell loss for these models. Thus, a single injection of MPTP (30 mg/kg) induces a 20-30% loss of DA neurons [16], a modified acute paradigm of 2 injections/day over 2 days leads to a 35% loss [62], and 4 injections kill approximately 50% of the neurons [28]. The subacute regimen over 8 days produces a 24% loss of DA neurons [63]. In addition, there is stereological evidence that the subacute protocol leads to a strong recovery of the DA neurons over time [95], suggesting that the toxic insult with this regimen is insufficient for permanent destruction of the nigrostriatal pathway.

The chronic MPTP (plus probenecid) regimen produces a rapid but more progressive loss of SN DA neurons when compared to other MPTP protocols [95]. Within a week after this regimen, 50% of SN DA neurons have been lost and up to 70-80% have disappeared by 3 weeks post-treatment; The latter loss can still be demonstrated 6 months later [15, 79, 95]. Thus, this chronic regimen provides a short preclinical ‘window’ following toxin administration for the introduction of neuroprotective strategies (table 1). Accompanying DA loss is a reduction in concentrations of dopamine and TH-immunopositive fibers throughout the dorsal striatum, with a sparing of the nucleus accumbens. Small granular inclusions that contain alpha-synuclein, have been seen in DA SN neurons and limbic cortical cells between 3 and 24 weeks post-MPTP treatment [88, 90].

Overall, the MPTP-treated mouse models are disappointing in that DA neurons die so rapidly and there is little progressive loss of the nigrostriatal DA pathway. Nevertheless, the pattern of DA terminal loss in the striatum replicates that of PD. Extra-nigral pathology has been demonstrated in reduced levels of monoamines other than dopamine [48, 131] and the inclusions in cortical regions [90].

In terms of motor deficits, the different MPTP protocols have also been disappointing [89]. The Rotarod and open field locomotion tests have been widely employed but are only effective if they are administered shortly after treatment when the mice are still intoxicated by MPTP. Mice tested later sometimes show hyperactivity and no deficit on the Rotarod [89]. Nevertheless, more sensitive measures, such as gait analysis, or the pole or grid tests, have been able to detect DA loss as low as 50% [89]. Unfortunately, motor deficits do not correlate well with the degree of DA neuronal loss, striatal DA levels or the dose of MPTP [101].

Intraventricular MPP+

MPP⁺, the toxic metabolite of MPTP and a Complex I inhibitor, is an excellent substrate for the DA transporter, which explains its selectivity towards DA neurons. Systemic administration of MPP⁺ does not damage central DA neurons, because it does not readily cross the blood brain barrier due to its charge. However, its direct infusion into the brain effectively destroys much of the DA nigrostriatal pathway. A chronic rat model has been developed which involves the continuous delivery of MPP⁺ for 28 days via an osmotic minipump that delivers the toxicant to the left lateral cerebral ventricle [137, 139]. This model is unilateral in order to avoid the moribund condition that arises with extensive

bilateral loss of DA neurons. This MPP+ treatment produces a dose-dependent, unilateral loss of striatal DA and TH on the side of the infusion. At low MPP+ doses (0.086 and 0.142 mg/kg/day), striatal DA is selectively reduced by 37% and 53%, respectively. While higher MPP+ doses (0.432 and 0.960 mg/kg/day) produce a greater DA loss (up to 90%), they also cause significant reductions in serotonin levels. At 0.142 mg/kg/day, there is a progressive loss of DA neurons. At 28 days after initiation of MPP+ treatment, SN DA cells are reduced by 35%; at 42 days, cell loss is further reduced to 65% in the ipsilateral side. On the contralateral side, DA cell number was similar in control animals and MPP+ animals evaluated at 28 days. At 42 days, in the contralateral side, there was a non-significant reduction in DA cell number (approximately 40%), findings, which indicate there may be delayed contralateral DA cell loss. These latter findings need to be further investigated because if a delay in contralateral DA loss occurs, this would better model the human condition in which unilateral motor deficits seen in the early stage of PD is replaced by bilateral deficits as the disease progresses. Also, at the latter time point, many surviving DA neurons are silver-stained, indicating ongoing degeneration. Other pathological findings include striatal and SN microglial activation and striatal inclusion bodies that immunoreact for alpha-synuclein and ubiquitin. One of the caveats is the apparent lack of inclusion bodies in the SN. However, upon ultrastructural examination of SN DA neurons, swollen and abnormal mitochondria with electron dense material are observed, reminiscent of defective mitochondria seen in other models and in cybrids from PD patients [37, 88, 121]. Whether SN inclusions develop with longer MPP+ exposures or survival times remains to be determined. No behavioral assessments were performed in these studies so we cannot correlate DA loss with motor deficits. While the model is technically challenging, it produces a reliable response with low

variation, thus making it appealing for testing neuroprotective strategies during the phase of toxic insult and ongoing degeneration, the stage at which PD patients present with the disease.

Systemic Rotenone

Several epidemiological studies link pesticide exposure to PD [3, 25]. Rotenone, a naturally occurring pesticide used in the environment, is a Complex I mitochondrial inhibitor that has been used to generate the first chronic PD model: rats receive rotenone via osmotic minipumps for up to 5 weeks, i.v. or s.c. [5, 53, 110]. Rotenone is lipophilic, readily crosses cell membranes and easily penetrates the blood-brain-barrier. At 2-3 mg/kg/day, it produces a loss of striatal DA terminals followed by progressive degeneration of SN DA neurons. Notably, dying DA neurons contain cytoplasmic inclusions, which like Lewy bodies, are immunopositive for alpha-synuclein and ubiquitin. Other pathological features include elevations in oxidative damage, microgliosis and increased iron deposits. Behaviorally, the rats display prominent motor deficits [36]. The progressive nature of degeneration and presence of neuronal inclusions are advantages of the rotenone model over more acute administration of other toxins. However, even with identical experimental conditions, rotenone causes either selective damage to DA neurons or more widespread cell loss [5, 110]. Thus, while the DA neurons may be most vulnerable to rotenone exposure, other unrelated populations can be damaged as well, and the high variability limits the utility of the model [36, 140]. An i.p. route of administration may circumvent these problems. Alam and Schmidt [2], using chronic daily i.p. injections of rotenone (1.5-2.5 mg/kg/day for 60 days), observed

reduced striatal DA content and TH immunoreactivity (immunoblots), and levodopa-responsive motor impairments. More recently, Greenamyre and colleagues have shown that rats treated i.p. (daily) with a 2.75-3.0 mg/kg dose, display other features of PD, including SN accumulation and aggregation of alpha-synuclein, microgliosis, iron accumulation, loss of enteric neurons and cardiac sympathetic denervation (Greenamyre, personal communication). These animals show less variability compared to the osmotic pump delivery paradigm, thus making this an attractive model for therapeutic testing in animals demonstrating early and late stages of parkinsonism (table 1).

Paraquat and Maneb

Other environmental toxins known to disrupt mitochondrial respiration and produce ROS have been systemically administered to produce mouse PD models (table 1; [96]).

Among these is paraquat (PQ), a herbicide that crosses the blood-brain-barrier. Its neurotoxicity can be attributed to redox cycling and ROS formation. Within cells, PQ is transported into mitochondria by a carrier-mediated process [21], where it is reduced by Complex I forming a PQ radical capable oxidatively damaging the mitochondria. Thus, whereas MPP⁺ and rotenone directly inhibit Complex I function, PQ indirectly disrupts mitochondrial function via intra-mitochondrial ROS formation through Complex I interactions with PQ. Various investigators have demonstrated small but significant losses of SN DA neurons with PQ [12, 36, 69, 85, 93] and up-regulation and aggregation of alpha-synuclein [29, 83]. However, studies have yet to demonstrate progressive DA cell loss or motor deficits.

Maneb (manganese ethylenebisadithiocarbamate), a fungicide that inhibits glutamate transport and disrupts DA uptake and release [125, 126], is generally co-administered with PQ subchronically to enhance toxicity. When combined with maneb (30mg/kg), PQ (10mg/kg) at 1-2 injections/week (3-6 weeks) destroys 50% of SN DA neurons in young mice [118]. In older mice (18 months of age), combined PQ/maneb treatment produces a more progressive DA cell loss, i.e. approximately 75% at 2 weeks and 88% at 12 weeks [117]. Studies in older rats have shown that they are very sensitive to the toxic effects of the combination PQ/maneb at the same doses used in younger mice [20, 102]. Loss of DA neurons, motor impairment and microgliosis, which are found in both young and old rats, mimic different stages of clinical PD. However, a potential disadvantage of PQ/maneb treatment for older rats is systemic lung toxicity, which can be lethal [102].

PD models: acute and chronic inflammation

Neuroinflammation is mediated predominately by microglia, the resident immuno-competent and phagocytic cells within the CNS. Microglia, representing 5-20% of brain cells [7, 26], exhibit, in their basal resting state, a ramified morphology that monitor the environment (reviewed in [133]). When activated, microglia undergo dramatic morphological changes, converting to an amoeboid state with enlarged cytoplasmic processes capable of phagocytosis. Activated cells also produce pro-inflammatory molecules such as chemokines, cytokines, nitric oxide and ROS used for clearing toxic debris [4, 7, 80]. The phagocytic activity is beneficial during neuronal development and in injury, as this process effectively removes cellular debris, but dysregulation or excessive activation, and ill-controlled ROS formation, can lead to an oxidative burden

for neurons. Microglial-induced inflammation can be sustained and progressive [41, 42, 86]. The observation that microgliosis persists for years in humans and non-human primates following acute exposure to MPTP [72, 86] indicates that the inflammatory response persists in the absence of continued exposure to the neurotoxicant, a feature important for understanding cell death in PD.

Acute Intracerebral LPS

Intracerebral injections of LPS (5 or 10 µg) into the cortex, hippocampus, striatum or SN of rats enhances the death of only SN DA neurons, possibly because microglial cell density in the SN is 4-5 times higher than in other regions [41, 52, 65]. LPS is now well established as an effective initiator of DA neurodegeneration. Acute intra-nigral or supra-nigral LPS injections (2 µg) produce a rapid activation of microglia (within 24 h) and loss of striatal dopamine (by 4 days) accompanied by loss of SN DA neurons (by 21 days) [13, 56]. While striatal dopamine is rapidly reduced, no further decline is seen up to 1 year, indicating a permanent lesion but a lack of progression [52]. Although acute LPS administration produces a rapid and intense microglial response, microglia morphology reverts to normal by 30 days, indicating a short-lived response and not a prolonged or progressive state of activation [56]. Rats exposed acutely to LPS rapidly lose TH-immunoreactive neurons in the SN and show unilateral behavioral deficits as evidenced by ipsiversive circling following amphetamine administration [56]. Others have seen a more progressive loss of TH-immunoreactive neurons months after a single acute insult [98].

Chronic Intracerebral LPS

To overcome the short-lived microglial response and develop a more progressive PD model, LPS has been administered chronically to rats. LPS is infused via stereotactically implanted cannulae just above the SN using osmotic minipumps [41]. This exposure (5 ng/h) for 2 weeks produces a rapid microglial activation (within 3 days) and signs of oxidative stress that persists for at least 8 weeks. The activation precedes DA cell death, which is not significant until 6 weeks into the study, but is progressive (approximately 10%, 40% and 60% at 4, 6 and 10 weeks, respectively, after initiation of exposure). While this model is attractive in that it presents with progressive DA cell loss, it remains to be determined if motor symptoms accompany the cell loss, alpha-synuclein-positive inclusions in DA neurons form or extra-nigral pathology occurs. Moreover, the techniques pose a technical challenge.

Acute Systemic LPS

A recent report describes the effects of a single systemic injection of LPS (5 mg/kg i.p.) in a mouse. Brain TNF α mRNA and protein rapidly increase (by 7,336% and 653%, respectively) within 1 hr of administration and remain elevated for 10 months [98]. Likewise, microglia in several brain regions (hippocampus, cortex, SN) become activated within a few hours of administration. However, DA cell loss is delayed but is progressive. Significant SN DA cell loss is not observed until 7 months of age (23% loss) with further reductions seen at 10 months of age (47% loss). Unfortunately, striatal DA changes and alpha-synuclein aggregates or other SN cell inclusion bodies have yet to

be investigated. Nonetheless, the studies indicate that a single exposure to a systemic inflammogen initiates a self-propagating response, which ultimately leads to the loss of SN DA neurons. Interestingly, progressive DA cell loss occurs in mice given a single systemic exposure to LPS, which contrasts with the lack of progressive DA neuron loss in rats provided with a single, acute, intra-nigral LPS infusion [13, 52, 56]. If findings in mice are confirmed, this model would be attractive, especially if inclusions form and behavior can be correlated with cell loss.

Intrauterine LPS

Carvey and colleagues have proposed that prenatal exposure to LPS not only creates a neuroinflammatory response but also disrupts the normal development of DA neurons. They studied the effects of prenatal LPS exposure on DA cell development and postnatal DA cell number in rats [77, 78]. In utero exposure to LPS following a single injection of the endotoxin into gravid female rats causes a significant (29%) reduction in striatal DA and 27% and 22% reduction in SN DA cell number in offspring killed at 21 days or 18 months, respectively, findings that suggest that prenatal infections could potentially be a risk factor for PD [76, 77]. Moreover, rotenone (1.25 mg/kg/day, 14 days, intrajugular) injected at 18 months of age to rats exposed prenatally to LPS, exerted a synergistic effect on DA cell loss. There was a significant reduction in SN DA neurons (39%), findings that suggest a pre-existing pro-inflammatory state can be a risk factor for environmental toxins [76]. Finally, the data demonstrate that exposures to different toxicants, separated by months or years, can synergize in their detrimental actions on DA neurons.

PD models: genetic manipulations

Three types of genetic models of PD have recently been developed. First, mouse models based on the deletion of genes important for the development or maintenance of DA neurons or their phenotype [55, 109, 113]. These mice exhibit DA cell loss at various times in their life, thus reproducing a cardinal feature of PD. However, they fail to reproduce the broad extra-nigral pathology and other pathological landmarks such as Lewy bodies. Furthermore, the relevance of these genetic mutations to PD is not fully established. Second, mouse or rat models based on expression or deletion of genes known to cause familial forms of PD [31]. Although these mutations are very rare, they point towards mechanisms that are most certainly related to PD in humans. The relevance of these specific genes or mutations to sporadic PD, however, is only clearly established for alpha-synuclein, the gene in which the first PD-causing mutations were discovered [19]. Finally, a third class of genetic models is based on virally mediated expression of genes or mutations known to cause familial PD, usually in nigrostriatal DA neurons [123]. These models produce a more acute form of the disease than transgenic or knock out animals. Nevertheless, they are valuable because they often exhibit neuronal loss, a feature that has been elusive in genetically engineered mice expressing PD-causing mutations.

Genetically engineered mice: mutations leading to nigrostriatal DA cell loss

Two models have achieved a progressive, post-natal loss of SN DA neurons:

1) *Pitx3* $-/-$ mice: These mice have a spontaneous mutation in the homeobox transcription factor Pitx3 and were originally identified based on a small eye phenotype (and blindness) and named aphakia mice. After the role of Pitx3 in DA development was identified, several groups discovered that these mice also lose nigrostriatal DA neurons early during post-natal development [55, 91, 111, 127]. Aphakia mice show behavioral deficits that are reversed by levodopa [55, 127]. Interestingly, mesolimbic DA neurons are resistant to Pitx3 loss, similar to what is observed in PD. The relevance of this mutation to sporadic PD remained elusive until recent evidence that polymorphism in the Pitx3 gene represents a risk factor for PD [39]. Nevertheless, the loss of DA neurons is the only PD feature reproduced in these mice; therefore, they may be useful to study survival factors for DA neurons or symptomatic treatments to counteract the consequences of striatal DA loss but can hardly be considered a model of the disease. Furthermore, they lack the characteristic progressive nature of sporadic PD, in which DA cell loss begins in adulthood.

2) *Engrailed knock-out (KO) mice*: Engrail 1 is primarily expressed in mesencephalic DA neurons, whereas engrail 2 is primarily expressed in cerebellum. To avoid compensation by one engrailed gene for the other, investigators generated engrail 1 $+/-$ on a background of engrail 2 $-/-$ (knocking out both forms of engrail is embryonic lethal) [109]. These mice show a progressive loss of nigrostriatal DA neurons but also cerebellar pathology, which limits their use in behavioral assays of nigrostriatal dysfunction. Another line that lacks one copy of engrail 1 with preserved engrail 2 shows more specific nigrostriatal DA cell loss without cerebellar pathology [113]. DA cell loss is progressive but it starts during late post-natal development, i.e. probably much earlier than in sporadic PD. These mice

show behavioral deficits, including marked affective disorders, a frequent symptom of PD. The search for the relevance of engrailed mutations to PD remains ongoing.

Genetically engineered mice that express mutations of familial PD

Five mutations (alpha-synuclein, Parkin, PINK1, DJ1, and LRRK2) have been linked to familial PD [50, 68].

1) Alpha-synuclein overexpressing mice: Single point mutations or gene multiplication of alpha-synuclein lead to familial forms of PD [74]. The latter indicates that increased levels of wild-type alpha-synuclein can cause PD. This establishes an important link with sporadic PD in which alpha-synuclein is not mutated but accumulates in Lewy bodies or neurites in a broad range of affected neurons, including but not limited to nigrostriatal DA neurons [47]. Many lines of mice expressing mutations in alpha-synuclein have been generated over the last decade [19]. They differ in the promoter used, which is critical in determining the relevance of the resulting line in modeling PD, and whether the transgene encodes wild-type or mutated alpha-synuclein. The TH promoter was used to reproduce the loss of catecholaminergic neurons found in PD. However, the restricted expression of the transgene does not mimic the broad alpha-synuclein pathology that characterizes the human disease. A different approach is to use a promoter that confers broad neuronal expression. The prion promoter has been particularly successful in generating models of amyotrophic lateral sclerosis because it drives high levels of transgenes in motoneurons [43, 75]. Accordingly, mice overexpressing alpha-synuclein under the prion promoter exhibit motor neuron pathology, which is different from PD. Therefore, these mice

provide information on mechanisms of alpha-synuclein-driven cell death *in vivo*, but they would not be useful to identify the specific, cell autonomous mechanisms in PD. Furthermore, the motor deficits cannot be attributed to nigrostriatal dysfunction or other parkinsonian symptoms.

Other promoters used to over-express alpha-synuclein in mice include PDGFbeta and Thy-1 [17, 84, 100, 128]. Both confer broad neural expression but the pattern of transgene expression varies [51]. The Thy-1 promoter drives higher levels of transgene expression in the SN pars compacta than the PDGFbeta promoter, thus better mimics the breadth of pathology observed in sporadic PD. Some lines using the Thy1 promoter display motor neuron pathology [128], but others do not, despite high levels of transgene expression [100]. The latter mice present progressive sensorimotor deficits starting as early as 2 months of age and worsening with age [33]. These deficits are detected with behavioral tests that are sensitive to nigrostriatal dysfunction [55], however they occur in the absence of DA cell loss, and accordingly, are not reversed by levodopa [34]. Therefore, these deficits do not correspond to the symptoms of parkinsonism observed in manifest PD but may represent early alterations in motor function that remain “subclinical” in patients. Indeed, these mice show olfactory and autonomic deficits similar to symptoms often observed before the onset of classical neurological symptoms in PD [32, 35]. In addition, they exhibit proteinase K resistant alpha-synuclein aggregates, which increase in size and become widespread with age (unpublished observations, [29]). With standard housing, these mice do not lose DA neurons up to 18 months of age. Nevertheless, the progressive motor deficits indicative of neuronal dysfunction, non-motor symptoms, and progressive pathological anomalies that are strongly reminiscent of early stages of PD, provide the opportunity to analyze the role of

alpha-synuclein accumulation in PD and to test novel therapeutic interventions to stop disease progression.

Few lines of alpha-synuclein transgenic mice show prominent loss of DA neurons, even though some show decreased striatal DA levels [84, 120]. One line expresses a doubly mutated alpha-synuclein, combining two mutations that lead to PD in humans, under the TH promoter [119]. Interestingly, another mouse, expressing a truncated form of alpha-synuclein, shows profound loss of DA but, disappointingly, this phenotype is present in young animals and does not increase with age, thus failing to provide a useful model for PD progression [130].

In conclusion, among the many lines of mice developed to mimic the alpha-synuclein pathology observed in sporadic PD, only a few have emerged that provide useful information despite some shortcomings. We are still lacking a model that reproduces both the broad pathology of PD and a robust progressive loss of nigrostriatal DA neurons. The information provided by existing models now informs further efforts to generate such model.

2) *Parkin, PINK1 and DJ1 KO mice*: Many mutations in the gene encoding parkin cause a significant portion of early onset familial PD [132]. Most of these mutations likely cause a loss of function in parkin, a E3 ubiquitin ligase, probably leading to proteasomal dysfunction [50]. One parkin mutation (Q311X) however causes DA cell loss in *Drosophila* in a dominant manner and PD may occur in some patients heterozygous for parkin mutations [68, 104].

Two separate lines of mice with exon3 mutations leading to a lack of protein expression show progressive sensorimotor dysfunction without DA cell loss [44, 57],

whereas one line with an exon7 deletion showed anomalies in paired-pulse inhibition and a non-progressive loss of NE neurons in the locus coeruleus [129]. Other lines showed no behavioral deficits [94], while others show non-motor deficits [140]. Exon3 deletion mice show evidence of oxidative stress in proteomics studies [92]. In contrast to these lines, a more recent model shows not only progressive motor dysfunction but also DA cell loss at late ages [82]. These mice are transgenic for Q311X parkin, suggesting a dominant effect of this mutation.

In flies, both parkin and PINK1 mutations cause similar alterations in mitochondria [27]. This phenotype, however, is not observed in mice. Nevertheless, PINK1 KO mice show a decrease in evoked DA release in the striatum and deficits in corticostriatal plasticity that are reversed by DA agonists, suggesting they are secondary to the decrease in evoked DA release [67]. Indeed, multiple observations suggest that deficits in DA release machinery may be a primary mechanism eventually leading to the SN DA cell demise [116]. Examining the progression of these pathological phenotypes in mice should provide insights into the progression of DA neurodegeneration in humans.

DJ1 mutations cause decreased resistance to oxidative stress in cells, flies, and mice [27]. The association of these mutations with recessive forms of familial PD supports a long suspected role for oxidative stress in PD pathophysiology [46]. DJ1 KO mice, however, have little phenotype and do not develop DA cell loss [135], although some lines show an increased sensitivity to PQ [136].

3) LRRK2 mutations: A late onset familial PD can be caused by a mutation in the gene that encodes a leucine-rich repeat kinase 2 (LRRK2) [40]. It appears that cell toxicity of

mutant LRRK2 is dependent on its kinase activity [112] and transgenic mouse models are currently being developed.

Viral delivery of genes related to PD-causing mutations

The lack of DA cell loss in most lines of genetically engineered mice expressing PD-causing mutations may be due to a number of factors, including the development of effective compensatory mechanisms. To overcome this problem, a number of models have been developed based on the acute delivery of virally expressed genes into the SN [123]. Because this requires stereotactic infusions, the rat has been most often used, although it is possible to adapt the technique to mice. After overexpression of alpha-synuclein either with a lentivirus or with an adeno-associated virus into the SN, rats develop a progressive loss of DA neurons and associated behavioral deficits [66, 81]. Thus, these models are more effective in modeling the hallmark nigrostriatal degeneration of PD than most currently available genetically engineered mice. However, the local delivery of the genes does not reproduce the extra-nigral pathology and does not model the progressive development of this pathology throughout the nervous system.

Conclusions

Over the past 3 decades, there has been impressive advances in creating rodent models that demonstrate the progressive nature of PD. No model is perfect, but rodents can demonstrate many pathophysiological features of PD and their use has increased our understanding of the mechanisms underlying this neurodegenerative disorder [22] and opened doors to exploration of neuroprotective and neurorestorative strategies [23].

Rodents have drawbacks, such as their short life span or their quadrupedal locomotion and very different behavioral repertoire that preclude replication of some, typical PD motor deficits [14, 89]. Nevertheless, toxin- and inflammation-induced models have been repeatedly refined and new transgenic mice developed, so that more ‘progressive’ rodent models are now available. For example, in terms of toxin models, the location (striatum rather than SN or MFB) for delivery of 6-OHDA seems to be quite important for slowing DA neuronal loss in the SN [105], and graded injections of this toxin can mimic preclinical or clinical stages [122]. Moreover, recently developed motor tests have demonstrated that the hemiparkinsonian rat can be an exceptional model of stepping, postural and balance deficits of PD [60]. The MPTP models are clearly the most widely employed but are disappointing in replicating PD symptoms, due to the lack of progressive cell death or correlated motor symptoms of PD. Nevertheless, these models have been very useful for exploring the molecular basis for mitochondrial dysfunction [22]. Intracerebroventricular (ICV) administration of MPP+, systemic daily injection of rotenone, or chronic ICV LPS produce progressive DA neuron loss and, in many cases, behavioral deficits that replicate those seen in PD [2, 41, 137, 139]. However, the latter approaches are all technically challenging.

Genetic models of PD have opened new perspectives for modeling and understanding the progression of PD but the advantages and disadvantages of each approach must be carefully considered. It is important to distinguish models that reproduce the progressive degeneration of nigrostriatal DA neurons from those that model disease progression in the whole organism. Genetic modeling of nigrostriatal degeneration complements toxin-induced neuronal loss by reproducing insults that are mechanistically linked to PD in humans. These models can provide useful information on stages of neurodegeneration, in

particular on the interplay between protective and detrimental mechanisms, which are likely to contribute to the late onset of the disease and the effect of aging, a main risk factor for PD. For example: does neurodegeneration require age-related failure in autophagy or the accumulation of mitochondrial mutations? Are defense mechanisms, such as anti-apoptotic or anti-oxidant genes upregulated prior to the onset of cell death? Finally, the growing number of models exhibiting DA cell loss due to genetic mutation not yet known to be associated with PD, point towards new avenues of research for genetic risk factors for the disease.

Few models so far reproduce the progression of extra-nigral pathology that characterizes PD and is present both in the pre-manifest (before the classical motor symptoms appear) and in the manifest phase of the disease. KO mice expressing mutations that cause recessive forms of familial PD have progressive behavioral deficits but do not show alpha-synuclein pathology as in sporadic PD. The closest models to sporadic PD so far are based on the over-expression of alpha-synuclein under a broadly expressed neural promoter such as Thy-1. Although they have insoluble alpha-synuclein inclusions, they fail to exhibit true Lewy bodies. Nevertheless, these mice show progressive sensorimotor deficits as well as decreased olfaction and autonomic dysfunction [19]. Because these behavioral deficits occur in the absence of DA cell loss these mice provide a model of pre-manifest PD but the absence of DA cell loss limits their use as a model of manifest PD. They show a broad pattern of alpha-synuclein aggregates that is reminiscent but not identical to the progressive pathological stages of PD. Clearly, the use of the endogenous alpha-synuclein promoter would be necessary to more faithfully reproduce this pattern, but high levels of transgene expression may need the use of bacterial artificial chromosome (BAC) technology. Based on the information

available from existing models, sophisticated genetic techniques such as specific expression or removal of the transgene in defined brain regions with Cre-Lox technologies, and the expression of highly pathological forms of alpha-synuclein, for example truncated and/or phosphorylated [18, 75, 120], should permit a more mechanistic analysis of PD pathology progression in a genetic animal model.

Table 1. Features of PD recapitulated by systemic or central administration of toxins

toxin	time to greatest DA cell loss	striatal loss of dopamine	advantages	caveats
6-OHDA, into nigra or MFB	42 h	dose-dependent loss of DA innervation	1) full DA depletion of nigrostriatal pathway 2) mimics late-stage PD; graded lesions mimic earlier stages 3) test therapeutic strategies	1) not progressive 2) resembles axotomy 3) no inclusions 4) no extra-nigral pathology
6-OHDA, into striatum	16 wks	circumscribed loss of tyrosine hydroxylase immunoreactivity at injection site	1) progressive DA cell loss 2) produces incomplete lesions that mimic PD	1) strong striatal glial reaction 2) no inclusions 3) no extra-nigral pathology
MPTP, acute	24 h	dorsal striatum with sparing of nucleus accumbens	1) inhibits complex I activity 2) striatal TH loss mimics PD	1) not progressive 2) no inclusions
MPTP, subacute/subchronic	24 h	dorsal striatum with sparing of nucleus accumbens	1) inhibits complex I activity 2) test neuro-protective regimens	1) not progressive 2) recovery of some DA neurons over time 2) no inclusions
MPTP, (probenecid) chronic	3 weeks post-treatment	dorsal striatum with sparing of nucleus accumbens	1) inhibits complex I activity 2) alpha-synuclein inclusions in DA neurons 3) extra-nigral pathology	1) initial death of DA neurons is rapid 2) inclusions do not resemble Lewy bodies
Paraquat (PQ) and maneb	Within 7 days	little or no measurable change in striatal DA innervation	1) combination of PQ and maneb is more effective for DA depletion than PQ alone	1) inconsistent results on DA loss 2) no inclusions 3) no extra-nigral pathology
Rotenone, chronic pump	36 days or longer	dose-dependent loss of TH in dorsal striatum with sparing of nucleus accumbens	1) inhibits complex I activity 2) progressive DA cell loss 3) alpha-synuclein inclusions in DA neurons 4) i.p. administration shows extra-nigral pathology	1) large variations in animal sensitivity 2) variation in motor response
MPP+,	42 days or longer	dorsal striatum with	1) inhibits complex I	1) no inclusions in

chronic pump		sparing of nucleus accumbens dose-dependent loss of DA innervation	activity 2) progressive DA cell loss 3) damaged mitochondria	DA neurons 2) motor deficits have yet to be demonstrated
LPS, acute into SN	21 days	dorsal striatum	1) activated microglia 2) rapid DA cell loss 3) permanent lesion	1) not progressive 2) no inclusions in DA neurons
LPS, chronic into SN	10 weeks	no data	1) chronic activation of microglia 2) progressive DA cell loss	1) no inclusions in DA neurons
LPS, acute systemic	7—10 months	no data	1) chronic activation of microglia 2) progressive DA cell loss	1) no inclusions in DA neurons

References

1. Aarsland D, Ballard CG, Halliday G (2004) Are Parkinson's disease with dementia and dementia with Lewy bodies the same entity? *J Geriatr Psychiatry Neurol* 17:137-145
2. Alam M, Schmidt WJ (2002) Rotenone destroys dopaminergic neurons and induces parkinsonian symptoms in rats. *Behav Brain Res* 136:317-324
3. Ascherio A, Chen H, Weisskopf MG, O'Reilly E, McCullough ML, Calle EE, Schwarzschild MA, Thun MJ (2006) Pesticide exposure and risk for Parkinson's disease. *Ann Neurol* 60:197-203
4. Barron KD (1995) The microglial cell. A historical review. *J Neurol Sci* 134 Suppl:57-68
5. Betarbet R, Sherer TB, MacKenzie G, Garcia-Osuna M, Panov AV, Greenamyre JT (2000) Chronic systemic pesticide exposure reproduces features of Parkinson's disease. *Nat Neurosci* 3:1301-1306
6. Bezard E, Dovero S, Bioulac B, Gross CE (1997) Kinetics of nigral degeneration in a chronic model of MPTP-treated mice. *Neurosci Lett* 234:47-50
7. Block ML, Zecca L, Hong JS (2007) Microglia-mediated neurotoxicity: uncovering the molecular mechanisms. *Nat Rev Neurosci* 8:57-69
8. Boka G, Anglade P, Wallach D, Javoy-Agid F, Agid Y, Hirsch EC (1994) Immunocytochemical analysis of tumor necrosis factor and its receptors in Parkinson's disease. *Neurosci Lett* 172:151-154
9. Braak H, Braak E, Yilmazer D, Schultz C, de Vos RA, Jansen EN (1995) Nigral and extranigral pathology in Parkinson's disease. *J Neural Transm Suppl* 46:15-31

10. Braak H, Del Tredici K, Rub U, de Vos RA, Jansen Steur EN, Braak E (2003) Staging of brain pathology related to sporadic Parkinson's disease. *Neurobiol Aging* 24:197-211
11. Breese GR, Traylor TD (1971) Depletion of brain noradrenaline and dopamine by 6-hydroxydopamine. *Br J Pharmacol* 42:88-99
12. Brooks AI, Chadwick CA, Gelbard HA, Cory-Slechta DA, Federoff HJ (1999) Paraquat elicited neurobehavioral syndrome caused by dopaminergic neuron loss. *Brain Res* 823:1-10
13. Castano A, Herrera AJ, Cano J, Machado A (1998) Lipopolysaccharide intranigral injection induces inflammatory reaction and damage in nigrostriatal dopaminergic system. *J Neurochem* 70:1584-1592
14. Cenci MA, Whishaw IQ, Schallert T (2002) Animal models of neurological deficits: how relevant is the rat? *Nat Rev Neurosci* 3:574-579
15. Chan CS, Guzman JN, Ilijic E, Mercer JN, Rick C, Tkatch T, Meredith GE, Surmeier DJ (2007) 'Rejuvenation' protects neurons in mouse models of Parkinson's disease. *Nature* 447:1081-1086
16. Chan P, Di Monte DA, Langston JW, Janson AM (1997) (+)MK-801 does not prevent MPTP-induced loss of nigral neurons in mice. *J Pharmacol Exp Ther* 280:439-446
17. Chandra S, Gallardo G, Fernandez-Chacon R, Schluter OM, Sudhof TC (2005) Alpha-synuclein cooperates with CSPalpha in preventing neurodegeneration. *Cell* 123:383-396

18. Chen L, Feany MB (2005) Alpha-synuclein phosphorylation controls neurotoxicity and inclusion formation in a *Drosophila* model of Parkinson disease. *Nat Neurosci* 8:657-663
19. Chesselet MF (2007) In vivo alpha-synuclein overexpression in rodents: A useful model of Parkinson's disease? *Exp Neurol* doi:10.1016/j.expneurol.2007.08.006
20. Cicchetti F, Lapointe N, Roberge-Tremblay A, Saint-Pierre M, Jimenez L, Ficke BW, Gross RE (2005) Systemic exposure to paraquat and maneb models early Parkinson's disease in young adult rats. *Neurobiol Dis* 20:360-371
21. Cocheme HM, Murphy MP (2007) Complex I is the major site of mitochondrial superoxide production by paraquat. *J Biol Chem* doi:10.1074/jbc.M708597200
22. Dauer W, Przedborski, S. (2003) Parkinson's disease: Mechanisms and models. *Neuron* 39:889-909
23. Dawson TM, Dawson VL (2002) Neuroprotective and neurorestorative strategies for Parkinson's disease. *Nat Neurosci* 5 Suppl:1058-1061
24. Dawson TM, Dawson VL (2003) Molecular pathways of neurodegeneration in Parkinson's disease. *Science* 302:819-822
25. Dick FD, De Palma G, Ahmadi A, Scott NW, Prescott GJ, Bennett J, Semple S, Dick S, Counsell C, Mozzoni P, Haites N, Wettinger SB, Mutti A, Otelea M, Seaton A, Soderkvist P, Felice A (2007) Environmental risk factors for Parkinson's disease and parkinsonism: the Geoparkinson study. *Occup Environ Med* 64:666-672
26. Dobrenis K (1998) Microglia in cell culture and in transplantation therapy for central nervous system disease. *Methods* 16:320-344

27. Dodson MW, Guo M (2007) Pink1, Parkin, DJ-1 and mitochondrial dysfunction in Parkinson's disease. *Curr Opin Neurobiol* 17:331-317
28. Du Y, Ma Z, Lin S, Dodel RC, Gao F, Bales KR, Triarhou LC, Chernet E, Perry KW, Nelson DL, Luecke S, Phebus LA, Bymaster FP, Paul SM (2001) Minocycline prevents nigrostriatal dopaminergic neurodegeneration in the MPTP model of Parkinson's disease. *Proc Natl Acad Sci USA* 98:14669-14674
29. Fernagut PO, Hutson CB, Fleming SM, Tetreaut NA, Salcedo J, Masliah E, Chesselet MF (2007) Behavioral and histopathological consequences of paraquat intoxication in mice: effects of alpha-synuclein over-expression. *Synapse* 61:991-1001
30. Fleming SM, Delville Y, Schallert T (2005) An intermittent, controlled-rate, slow progressive degeneration model of Parkinson's disease: antiparkinson effects of Sinemet and protective effects of methylphenidate. *Behav Brain Res* 156:201-2213
31. Fleming SM, Fernagut PO, Chesselet MF (2005) Genetic mouse models of parkinsonism: strengths and limitations. *NeuroRx* 2:495-503
32. Fleming SM, Jordan MD, Masliah E, Chesselet MF, Roos KP (2007) Alterations in baroreceptor function in transgenic mice overexpressing human alpha synuclein. *Neurosci Abst* 33:50.59
33. Fleming SM, Salcedo J, Fernagut PO, Rockenstein E, Masliah E, Levine MS, Chesselet MF (2004) Early and progressive sensorimotor anomalies in mice overexpressing wild-type human alpha-synuclein. *J Neurosci* 24:9434-9440
34. Fleming SM, Salcedo J, Hutson CB, Rockenstein E, Masliah E, Levine MS, Chesselet MF (200) Behavioral effects of dopaminergic agonists in transgenic

- mice overexpressing human wildtype alpha-synuclein. *Neuroscience* 142:1245-1253
35. Fleming SM, Tetreault NA, Masliah E, Chesselet MF (2006) Alterations in olfactory function in transgenic mice overexpressing human wildtype alpha-synuclein. *Neurosci Abst* 32:75.79
 36. Fleming SM, Zhu C, Fernagut PO, Mehta A, DiCarlo CD, Seaman RL, Chesselet MF (2004) Behavioral and immunohistochemical effects of chronic intravenous and subcutaneous infusions of varying doses of rotenone. *Exp Neurol* 187:418-429
 37. Fornai F, Schluter OM, Lenzi P, Gesi M, Ruffoli R, Ferrucci M, Lazzeri G, Busceti CL, Pontarelli F, Battaglia G, Pellegrini A, Nicoletti F, Ruggieri S, Paparelli A, Sudhof TC (2005) Parkinson-like syndrome induced by continuous MPTP infusion: convergent roles of the ubiquitin-proteasome system and alpha-synuclein. *Proc Natl Acad Sci USA* 102:3413-3418
 38. Fornai F, Vaglini, F., Maggio, R., Bonuccelli U., Corsini, G.U. (1997) Species differences in the role of excitatory amino acids in experimental parkinsonism. *Neurosci Biobehav Rev* 21:401-415
 39. Fuchs J, Mueller JC, Lichtner P, Schulte C, Munz M, Berg D, Wullner U, Illig T, Sharma M, Gasser T (2007) The transcription factor PITX3 is associated with sporadic Parkinson's disease. *Neurobiol Aging* doi:10.1016/j.neurobiolaging.2007.08.014
 40. Funayama M, Hasegawa K, Kowa H, Saito M, Tsuji S, Obata F (2002) A new locus for Parkinson's disease (PARK8) maps to chromosome 12p11.2-q13.1. *Ann Neurol* 51:296-301

41. Gao HM, Jiang J, Wilson B, Zhang W, Hong JS, Liu B (2002) Microglial activation-mediated delayed and progressive degeneration of rat nigral dopaminergic neurons: relevance to Parkinson's disease. *J Neurochem* 81:1285-1297
42. Gao HM, Liu B, Hong JS (2003) Critical role for microglial NADPH oxidase in rotenone-induced degeneration of dopaminergic neurons. *J Neurosci* 23:6181-6187
43. Gispert S, Del Turco D, Garrett L, Chen A, Bernard DJ, Hamm-Clement J, Korf HW, Deller T, Braak H, Auburger G, Nussbaum RL (2003) Transgenic mice expressing mutant A53T human alpha-synuclein show neuronal dysfunction in the absence of aggregate formation. *Mol Cell Neurosci* 24:419-429
44. Goldberg MS, Fleming SM, Palacino JJ, Cepeda C, Lam HA, Bhatnagar A, Meloni EG, Wu N, Ackerson LC, Klapstein GJ, Gajendiran M, Roth BL, Chesselet MF, Maidment NT, Levine MS, Shen J (2003) Parkin-deficient mice exhibit nigrostriatal deficits but not loss of dopaminergic neurons. *J Biol Chem* 278:43628-43635
45. Grant RJ, Clarke PB (2002) Susceptibility of ascending dopamine projections to 6-hydroxydopamine in rats: effect of hypothermia. *Neuroscience* 115:1281-1294
46. Greenamyre JT, Hastings TG (2004) Biomedicine. Parkinson's--divergent causes, convergent mechanisms. *Science* 304:1120-1122
47. Halliday GM, Del Tredici K, Braak H (2006) Critical appraisal of brain pathology staging related to presymptomatic and symptomatic cases of sporadic Parkinson's disease. *J Neural Transm Suppl*:99-103

48. Hallman H, Lange J, Olson L, Stromberg I, Jonsson G (1985) Neurochemical and histochemical characterization of neurotoxic effects of 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine on brain catecholamine neurones in the mouse. *J Neurochem* 44:117-127
49. Harding AJ, Halliday GM (2001) Cortical Lewy body pathology in the diagnosis of dementia. *Acta Neuropathol* 102:355-363
50. Hardy J, Cai H, Cookson MR, Gwinn-Hardy K, Singleton A (2006) Genetics of Parkinson's disease and parkinsonism. *Ann Neurol* 60:389-398
51. Hashimoto M, Rockenstein E, Masliah E (2003) Transgenic models of alpha-synuclein pathology: past, present, and future. *Ann NY Acad Sci* 991:171-188
52. Herrera AJ, Castano A, Venero JL, Cano J, Machado A (2000) The single intranigral injection of LPS as a new model for studying the selective effects of inflammatory reactions on dopaminergic system. *Neurobiol Dis* 7:429-447
53. Hoglinger GU, Feger J, Prigent A, Michel PP, Parain K, Champy P, Ruberg M, Oertel WH, Hirsch EC (2003) Chronic systemic complex I inhibition induces a hypokinetic multisystem degeneration in rats. *J Neurochem* 84:491-502
54. Hunot S, Dugas N, Faucheux B, Hartmann A, Tardieu M, Debre P, Agid Y, Dugas B, Hirsch EC (1999) FcepsilonRII/CD23 is expressed in Parkinson's disease and induces, in vitro, production of nitric oxide and tumor necrosis factor-alpha in glial cells. *J Neurosci* 19:3440-3447
55. Hwang DY, Fleming SM, Ardayfio P, Moran-Gates T, Kim H, Tarazi FI, Chesselet MF, Kim KS (2005) 3,4-dihydroxyphenylalanine reverses the motor deficits in Pitx3-deficient aphakia mice: behavioral characterization of a novel genetic model of Parkinson's disease. *J Neurosci* 25:2132-2137

56. Iravani MM, Leung CC, Sadeghian M, Haddon CO, Rose S, Jenner P (2005) The acute and the long-term effects of nigral lipopolysaccharide administration on dopaminergic dysfunction and glial cell activation. *Eur J Neurosci* 22:317-330
57. Itier JM, Ibanez P, Mena MA, Abbas N, Cohen-Salmon C, Bohme GA, Laville M, Pratt J, Corti O, Pradier L, Ret G, Joubert C, Periquet M, Araujo F, Negroni J, Casarejos MJ, Canals S, Solano R, Serrano A, Gallego E, Sanchez M, Deneffe P, Benavides J, Tremp G, Rooney TA, Brice A, Garcia de Yebenes J (2003) Parkin gene inactivation alters behaviour and dopamine neurotransmission in the mouse. *Hum Mol Genet* 12:2277-22791
58. Javoy F, Sotelo C, Herbet A, Agid Y (1976) Specificity of dopaminergic neuronal degeneration induced by intracerebral injection of 6-hydroxydopamine in the nigrostriatal dopamine system. *Brain Res* 102:201-215
59. Jeon BS, Jackson-Lewis V, Burke RE (1995) 6-Hydroxydopamine lesion of the rat substantia nigra: time course and morphology of cell death. *Neurodegeneration* 4:131-137
60. Johnston RE, Schallert T, Becker JB (1999) Akinesia and postural abnormality after unilateral dopamine depletion. *Behav Brain Res* 104:189-196
61. Jonsson G (1980) Chemical neurotoxins as denervation tools in neurobiology. *Annu Rev Neurosci* 3:169-187
62. Joyce JN, Woolsey C, Ryoo H, Borwege S, Hagner D (2004) Low dose pramipexole is neuroprotective in the MPTP mouse model of Parkinson's disease, and downregulates the dopamine transporter via the D3 receptor. *BMC Biol* 2:22
doi:10.1186/1741-7007-2-22

63. Karunakaran S, Diwakar L, Saeed U, Agarwal V, Ramakrishnan S, Iyengar S, Ravindranath V (2007) Activation of apoptosis signal regulating kinase 1 (ASK1) and translocation of death-associated protein, Daxx, in substantia nigra pars compacta in a mouse model of Parkinson's disease: protection by alpha-lipoic acid. *FASEB J* 21:2226-2236
64. Kennedy JL, Farrer LA, Andreassen NC, Mayeux R, St George-Hyslop P (2003) The genetics of adult-onset neuropsychiatric disease: complexities and conundra? *Science* 302:822-826
65. Kim WG, Mohny RP, Wilson B, Jeohn GH, Liu B, Hong JS (2000) Regional difference in susceptibility to lipopolysaccharide-induced neurotoxicity in the rat brain: role of microglia. *J Neurosci* 20:6309-6316
66. Kirik D, Rosenblad C, Burger C, Lundberg C, Johansen TE, Muzyczka N, Mandel RJ, Bjorklund A (2002) Parkinson-like neurodegeneration induced by targeted overexpression of alpha-synuclein in the nigrostriatal system. *J Neurosci* 22:2780-2791
67. Kitada T, Pisani A, Porter DR, Yamaguchi H, Tscherter A, Martella G, Bonsi P, Zhang C, Pothos EN, Shen J (2007) Impaired dopamine release and synaptic plasticity in the striatum of PINK1-deficient mice. *Proc Natl Acad Sci USA* 104:11441-11446
68. Klein C, Lohmann-Hedrich K, Rogaeva E, Schlossmacher MG, Lang AE (2007) Deciphering the role of heterozygous mutations in genes associated with parkinsonism. *Lancet Neurol* 6:652-662
69. Kuter K, Smialowska M, Wieronska J, Zieba B, Wardas J, Pietraszek M, Nowak P, Biedka I, Roczniak W, Konieczny J, Wolfarth S, Ossowska K (2007) Toxic

- influence of subchronic paraquat administration on dopaminergic neurons in rats. *Brain Res* 1155:196-207
70. Lane E, Dunnett S (2007) Animal models of Parkinson's disease and L-dopa induced dyskinesia: How close are we to the clinic? *Psychopharmacology (Berl)* doi:10.1007/s00213-007-0931-8
 71. Lane EL, Cheetham SC, Jenner P (2006) Does contraversive circling in the 6-OHDA-lesioned rat indicate an ability to induce motor complications as well as therapeutic effects in Parkinson's disease? *Exp Neurol* 197:284-290
 72. Langston JW, Forno LS, Tetud J, Reeves AG, Kaplan JA, Karluk D (1999) Evidence of active nerve cell degeneration in the substantia nigra of humans years after 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine exposure. *Ann Neurol* 46:598-605
 73. Lau YS, Trobough KL, Crampton JM, Wilson JA (1990) Effects of probenecid on striatal dopamine depletion in acute and long-term 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP)-treated mice. *Gen Pharmacol* 21:181-187
 74. Lee VM, Trojanowski JQ (2006) Mechanisms of Parkinson's disease linked to pathological alpha-synuclein: new targets for drug discovery. *Neuron* 52:33-38
 75. Li W, West N, Colla E, Pletnikova O, Troncoso JC, Marsh L, Dawson TM, Jakala P, Hartmann T, Price DL, Lee MK (2005) 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* 102:2162-2167

76. Ling Z, Chang QA, Tong CW, Leurgans SE, Lipton JW, Carvey PM (2004) Rotenone potentiates dopamine neuron loss in animals exposed to lipopolysaccharide prenatally. *Exp Neurol* 190:373-383
77. Ling Z, Gayle DA, Ma SY, Lipton JW, Tong CW, Hong JS, Carvey PM (2002) In utero bacterial endotoxin exposure causes loss of tyrosine hydroxylase neurons in the postnatal rat midbrain. *Mov Disord* 17:116-124
78. Ling Z, Zhu Y, Tong C, Snyder JA, Lipton JW, Carvey PM (2006) Progressive dopamine neuron loss following supra-nigral lipopolysaccharide (LPS) infusion into rats exposed to LPS prenatally. *Exp Neurol* 199:499-512
79. Liss B, Haeckel O, Wildmann J, Miki T, Seino S, Roeper J (2005) K-ATP channels promote the differential degeneration of dopaminergic midbrain neurons. *Nat Neurosci* 8:1742-1751
80. Liu B (2006) Modulation of microglial pro-inflammatory and neurotoxic activity for the treatment of Parkinson's disease. *Aaps J* 8:E606-621
81. Lo Bianco C, Ridet JL, Schneider BL, Deglon N, Aebischer P (2002) alpha-synucleinopathy and selective dopaminergic neuron loss in a rat lentiviral-based model of Parkinson's disease. *Proc Natl Acad Sci USA* 99:10813-10818
82. Lu X, Fleming SM, Chesselet MF, Yang WX (2006) A novel BAC transgenic mouse model of PD overexpressing human mutant parkin in dopaminergic neurons. *Neurosci Abst* 32:612.3
83. Manning-Bog AB, McCormack AL, Li J, Uversky VN, Fink AL, Di Monte DA (2002) The herbicide paraquat causes up-regulation and aggregation of alpha-synuclein in mice: paraquat and alpha-synuclein. *J Biol Chem* 277:1641-1644

84. Masliah E, Rockenstein E, Veinbergs I, Mallory M, Hashimoto M, Takeda A, Sagara Y, Sisk A, Mucke L (2000) Dopaminergic loss and inclusion body formation in alpha-synuclein mice: implications for neurodegenerative disorders. *Science* 287:1265-1269
85. McCormack AL, Thiruchelvam M, Manning-Bog AB, Thiffault C, Langston JW, Cory-Slechta DA, Di Monte DA (2002) Environmental risk factors and Parkinson's disease: selective degeneration of nigral dopaminergic neurons caused by the herbicide paraquat. *Neurobiol Dis* 10:119-127
86. McGeer PL, Schwab C, Parent A, Doudet D (2003) Presence of reactive microglia in monkey substantia nigra years after 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine administration. *Ann Neurol* 54:599-604
87. McGeer PL, Yasojima K, McGeer EG (2001) Inflammation in Parkinson's disease. *Adv Neurol* 86:83-89
88. Meredith GE, Halliday, G.M., Totterdell, S. (2004) A critical review of the development and importance of proteinaceous aggregates in animal models of Parkinson's disease: New insights into Lewy body formation. *Parkinsonism Relat Disord* 10:191-202
89. Meredith GE, Kang UJ (2006) Behavioral models of Parkinson's disease in rodents: a new look at an old problem. *Mov Disord* 21:1595-1606
90. Meredith GE, Totterdell S, Petroske E, Santa Cruz K, Callison RC, Jr., Lau YS (2002) Lysosomal malfunction accompanies alpha-synuclein aggregation in a progressive mouse model of Parkinson's disease. *Brain Res* 956:156-165

91. Nunes I, Tovmasian LT, Silva RM, Burke RE, Goff SP (2003) Pitx3 is required for development of substantia nigra dopaminergic neurons. *Proc Natl Acad Sci USA* 100:4245-4250
92. Palacino JJ, Sagi D, Goldberg MS, Krauss S, Motz C, Wacker M, Klose J, Shen J (2004) Mitochondrial dysfunction and oxidative damage in parkin-deficient mice. *J Biol Chem* 279:18614-18622
93. Peng J, Stevenson FF, Doctrow SR, Andersen JK (2005) Superoxide dismutase/catalase mimetics are neuroprotective against selective paraquat-mediated dopaminergic neuron death in the substantia nigra: implications for Parkinson disease. *J Biol Chem* 280:29194-29198
94. Perez FA, Palmiter RD (2005) Parkin-deficient mice are not a robust model of parkinsonism. *Proc Natl Acad Sci USA* 102:2174-2179
95. Petroske E, Meredith GE, Callen S, Totterdell S, Lau YS (2001) Mouse model of Parkinsonism: a comparison between subacute MPTP and chronic MPTP/probenecid treatment. *Neuroscience* 106:589-601
96. Przedborski S, Ischiropoulos H (2005) Reactive oxygen and nitrogen species: weapons of neuronal destruction in models of Parkinson's disease. *Antioxid Redox Signal* 7:685-693
97. Przedborski S, Levivier M, Jiang H, Ferreira M, Jackson-Lewis V, Donaldson D, Togasaki DM (1995) Dose-dependent lesions of the dopaminergic nigrostriatal pathway induced by intrastriatal injection of 6-hydroxydopamine. *Neuroscience* 67:631-647

98. Qin L, Wu X, Block ML, Liu Y, Breese GR, Hong JS, Knapp DJ, Crews FT (2007) Systemic LPS causes chronic neuroinflammation and progressive neurodegeneration. *Glia* 55:453-462
99. Reavill C, Jenner P, Marsden CD (1983) Differentiation of dopamine agonists using drug-induced rotation in rats with unilateral or bilateral 6-hydroxydopamine destruction of ascending dopamine pathways. *Biochem Pharmacol* 32:865-870
100. Rockenstein E, Mallory M, Hashimoto M, Song D, Shults CW, Lang I, Masliah E (2002) Differential neuropathological alterations in transgenic mice expressing alpha-synuclein from the platelet-derived growth factor and Thy-1 promoters. *J Neurosci Res* 68:568-578
101. Rousselet E, Joubert C, Callebert J, Parain K, Tremblay L, Orieux G, Launay JM, Cohen-Salmon C, Hirsch EC (2003) Behavioral changes are not directly related to striatal monoamine levels, number of nigral neurons, or dose of parkinsonian toxin MPTP in mice. *Neurobiol Dis* 14:218-228
102. Saint-Pierre M, Tremblay ME, Sik A, Gross RE, Cicchetti F (2006) Temporal effects of paraquat/maneb on microglial activation and dopamine neuronal loss in older rats. *J Neurochem* 98:760-772
103. Saner A, Thoenen H (1971) Model experiments on the molecular mechanism of action of 6-hydroxydopamine. *Mol Pharmacol* 7:147-154
104. Sang TK, Chang HY, Lawless GM, Ratnaparkhi A, Mee L, Ackerson LC, Maidment NT, Krantz DE, Jackson GR (2007) A *Drosophila* model of mutant human parkin-induced toxicity demonstrates selective loss of dopaminergic neurons and dependence on cellular dopamine. *J Neurosci* 27:981-992

105. Sauer H, Oertel WH (1994) Progressive degeneration of nigrostriatal dopamine neurons following intrastriatal terminal lesions with 6-hydroxydopamine: a combined retrograde tracing and immunocytochemical study in the rat. *Neuroscience* 59:401-415
106. Schallert T, Fleming SM, Leasure JL, Tillerson JL, Bland ST (2000) CNS plasticity and assessment of forelimb sensorimotor outcome in unilateral rat models of stroke, cortical ablation, parkinsonism and spinal cord injury. *Neuropharmacology* 39:777-787
107. Schapira AH (1993) Mitochondrial complex I deficiency in Parkinson's disease. *Adv Neurol* 60:288-291
108. Sedelis M, Hofele, KI., Auburger, G.W., Morgan, S., Huston, J.P., Schwarting, R.K. (2000) MPTP susceptibility in the mouse: behavioral, neurochemical and histological analysis of gender and strain differences. *Behav Genet* 30:171-182
109. Sgado P, Alberi L, Gherbassi D, Galasso SL, Ramakers GM, Alavian KN, Smidt MP, Dyck RH, Simon HH (2006) Slow progressive degeneration of nigral dopaminergic neurons in postnatal Engrailed mutant mice. *Proc Natl Acad Sci USA* 103:15242-15247
110. Sherer TB, Kim JH, Betarbet R, Greenamyre JT (2003) Subcutaneous rotenone exposure causes highly selective dopaminergic degeneration and alpha-synuclein aggregation. *Exp Neurol* 179:9-16
111. Smidt MP, Smits SM, Bouwmeester H, Hamers FP, van der Linden AJ, Hellemons AJ, Graw J, Burbach JP (2004) Early developmental failure of substantia nigra dopamine neurons in mice lacking the homeodomain gene Pitx3. *Development* 131:1145-1155

112. Smith WW, Pei Z, Jiang H, Dawson VL, Dawson TM, Ross CA (2006) Kinase activity of mutant LRRK2 mediates neuronal toxicity. *Nat Neurosci* 9:1231-3
113. Sonnier L, Le Pen G, Hartmann A, Bizot JC, Trovero F, Krebs MO, Prochiantz A (2007) Progressive loss of dopaminergic neurons in the ventral midbrain of adult mice heterozygote for Engrailed1. *J Neurosci* 27:1063-1071
114. Sonsalla PK, Heikkila, R.E. (1986) The influence of dose and dosing interval on MPTP-induced dopaminergic neurotoxicity in mice. *Eur J Pharmacol* 129:339-345
115. Stanic D, Finkelstein DI, Bourke DW, Drago J, Horne MK (2003) Timecourse of striatal re-innervation following lesions of dopaminergic SNpc neurons of the rat. *Eur J Neurosci* 18:1175-1188
116. Sulzer D (2007) Multiple hit hypotheses for dopamine neuron loss in Parkinson's disease. *Trends Neurosci* 30:244-250
117. Thiruchelvam M, McCormack A, Richfield EK, Baggs RB, Tank AW, Di Monte DA, Cory-Slechta DA (2003) Age-related irreversible progressive nigrostriatal dopaminergic neurotoxicity in the paraquat and maneb model of the Parkinson's disease phenotype. *Eur J Neurosci* 18:589-600
118. Thiruchelvam M, Richfield EK, Baggs RB, Tank AW, Cory-Slechta DA (2000) The nigrostriatal dopaminergic system as a preferential target of repeated exposures to combined paraquat and maneb: implications for Parkinson's disease. *J Neurosci* 20:9207-9214
119. Thiruchelvam MJ, Powers JM, Cory-Slechta DA, Richfield EK (2004) Risk factors for dopaminergic neuron loss in human alpha-synuclein transgenic mice. *Eur J Neurosci* 19:845-854

120. Tofaris GK, Garcia Reitbock P, Humby T, Lambourne SL, O'Connell M, Ghetti B, Gossage H, Emson PC, Wilkinson LS, Goedert M, Spillantini MG (2006) 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* 26:3942-3950
121. Trimmer PA, Swerdlow RH, Parks JK, Keeney P, Bennett JP, Jr., Miller SW, Davis RE, Parker WD, Jr. (2000) Abnormal mitochondrial morphology in sporadic Parkinson's and Alzheimer's disease cybrid cell lines. *Exp Neurol* 162:37-50
122. Truong L, Allbutt H, Kassiou M, Henderson JM (2006) Developing a preclinical model of Parkinson's disease: a study of behaviour in rats with graded 6-OHDA lesions. *Behav Brain Res* 169:1-9
123. Ulusoy A, Bjorklund T, Hermening S, Kirik D (2007) In vivo gene delivery for development of mammalian models for Parkinson's disease. *Exp Neurol*
124. Ungerstedt U, Arbuthnott GW (1970) Quantitative recording of rotational behavior in rats after 6-hydroxy-dopamine lesions of the nigrostriatal dopamine system. *Brain Res* 24:485-493
125. Vaccari A, Ferraro L, Saba P, Ruiu S, Mocci I, Antonelli T, Tanganelli S (1998) Differential mechanisms in the effects of disulfiram and diethyldithiocarbamate intoxication on striatal release and vesicular transport of glutamate. *J Pharmacol Exp Ther* 285:961-967
126. Vaccari A, Saba PL, Ruiu S, Collu M, Devoto P (1996) Disulfiram and diethyldithiocarbamate intoxication affects the storage and release of striatal dopamine. *Toxicol Appl Pharmacol* 139:102-108

127. van den Munckhof P, Luk KC, Ste-Marie L, Montgomery J, Blanchet PJ, Sadikot AF, Drouin J (2003) Pitx3 is required for motor activity and for survival of a subset of midbrain dopaminergic neurons. *Development* 130:2535-2542
128. 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 (2000) Neuropathology in mice expressing human alpha-synuclein. *J Neurosci* 20:6021-6029
129. Von Coelln R, Thomas B, Savitt JM, Lim KL, Sasaki M, Hess EJ, Dawson VL, Dawson TM (2004) Loss of locus coeruleus neurons and reduced startle in parkin null mice. *Proc Natl Acad Sci USA* 101:10744-10749
130. Wakamatsu M, Ishii A, Iwata S, Sakagami J, Ukai Y, Ono M, Kanbe D, Muramatsu SI, Kobayashi K, Iwatsubo T, Yoshimoto M (2006) Selective loss of nigral dopamine neurons induced by overexpression of truncated human alpha-synuclein in mice. *Neurobiol Aging* doi:10.1016/j.neurobiolaging.2006.11.017
131. Wallace RA, Boldry R, Schmittgen T, Miller D, Uretsky N (1984) Effect of 1-methyl-4-phenyl-1,2,3,6 tetrahydropyridine (MPTP) on monoamine neurotransmitters in mouse brain & heart. *Life Sci* 35:285-291
132. West AB, Maidment NT (2004) Genetics of parkin-linked disease. *Hum Genet* 114:327-336
133. Whitton PS (2007) Inflammation as a causative factor in the aetiology of Parkinson's disease. *Br J Pharmacol* 150:963-976
134. Xu Z, Cawthon D, McCastlain KA, Slikker W, Jr., Ali SF (2005) Selective alterations of gene expression in mice induced by MPTP. *Synapse* 55:45-51

135. Yamaguchi H, Shen J (2007) Absence of dopaminergic neuronal degeneration and oxidative damage in aged DJ-1-deficient mice. *Mol Neurodegener* 2:10.doi.1186/1750-1326-2-10
136. Yang W, Chen L, Ding Y, Zhuang X, Kang UJ (2007) Paraquat induces dopaminergic dysfunction and proteasome impairment in DJ-1-deficient mice. *Hum Mol Genet* 16:2900-2910
137. Yazdani U, German DC, Liang CL, Manzino L, Sonsalla PK, Zeevalk GD (2006) Rat model of Parkinson's disease: Chronic central delivery of 1-methyl-4-phenylpyridinium (MPP+). *Exp Neurol* 200:172-183
138. Zahm DS (1991) Compartments in rat dorsal and ventral striatum revealed following injection of 6-hydroxydopamine into the ventral mesencephalon. *Brain Res* 552:164-169
139. Zeevalk GD, Manzino L, Sonsalla PK, Bernard LP (2007) Characterization of intracellular elevation of glutathione (GSH) with glutathione monoethyl ester and GSH in brain and neuronal cultures: relevance to Parkinson's disease. *Exp Neurol* 203:512-520
140. Zhu XR, Maskri L, Herold C, Bader V, Stichel CC, Gunturkun O, Lubbert H (2007) Non-motor behavioural impairments in parkin-deficient mice. *Eur J Neurosci* 26:1902-1911