Published in final edited form as:

Neuroscience. 2008 September 9; 155(4): 1174–1194. doi:10.1016/j.neuroscience.2008.06.064.

Distinct mechanisms of 1-methyl-4-phenyl-1,2,3,6-tetrahydropyrimidine resistance revealed by transcriptome mapping in mouse striatum

Roberto Pattarini^{1,*}, Yongqi Rong^{1,*}, Chunxu Qu², and James I. Morgan¹

¹Department of Developmental Neurobiology, St. Jude Children's Research Hospital, Memphis, TN, 38105

²Hartwell Center for Bioinformatics and Biotechnology, St. Jude Children's Research Hospital, Memphis, TN, 38105

Abstract

The etiology of idiopathic Parkinson's disease is thought to involve interplay between environmental factors and predisposing genetic traits, although the identification of genetic risk factors remain elusive. The neurotoxicant, 1-methyl-4-phenyl-1,2,3,6-tetrahydropyrimidine (MPTP) produces parkinsonian-like symptoms and pathology in mice and humans. As sensitivity to MPTP is genetically determined in mice this provides an opportunity to identify genes and biological mechanisms that modify the response to an exogenous agent that produces a Parkinson's disease-like condition. MPTP primarily targets dopaminergic nerve terminals in the striatum and elicits changes in striatal gene expression. Therefore, we used Affymetrix® and real-time PCR technology to characterize temporal mRNA changes in striatum in response to MPTP in genetically MPTP-sensitive, C57BL/6J, and MPTP-resistant Swiss Webster and Bax^{-/-} mice. We identified three phases of mRNA expression changes composed of largely distinct gene sets. An early response (5 hours) occurred in all strains of mice and multiple brain regions. In contrast, intermediate (24 hours) and late (72 hours) phases were striatum specific and much reduced in Swiss Webster, indicating these genes contribute and/or are responsive to MPTP-induced pathology. However, Bax^{-/-} mice have robust intermediate responses. We propose a model in which the acute entry of MPP⁺ into dopaminergic nerve terminals damages them but is insufficient per se to kill the neurons. Rather, we suggest that the compromised nerve terminals elicit longer lasting transcriptional responses in surrounding cells involving production of molecules that feedback on the terminals to cause additional damage that results in cell death. In Swiss Webster, resistance lies upstream in the cascade of events triggered by MPTP and uncouples the acute events elicited by MPTP from the damaging secondary responses. In contrast, in Bax^{-/-} mice resistance lies downstream in the cascade and suggests enhanced tolerance to the secondary insult rather than its attenuation.

Correspondence to: James I. Morgan, Department of Developmental Neurobiology, St. Jude Children's Research Hospital, 332 North Lauderdale Street, Danny Thomas Research Tower, Room D2025E, Mail Stop 323, Memphis, TN, 38105-2794 USA. Tel:

+1-901-495-2256; Fax: +1-901-495-3143. Email: jim.morgan@stjude.org.

*Both authors contributed equally to this article

Section Editor (molecular): Dr. Werner Sieghart, Brain research Institute, University of Vienna, Division of Biochemistry and Molecular Biology, Spitalgasse 4, A-1090 Vienna, Austria

Publisher's Disclaimer: This is a PDF file of an unedited manuscript that has been accepted for publication. As a service to our customers we are providing this early version of the manuscript. The manuscript will undergo copyediting, typesetting, and review of the resulting proof before it is published in its final citable form. Please note that during the production process errors may be discovered which could affect the content, and all legal disclaimers that apply to the journal pertain.

Keywords

C57BL/6J mice; Swiss Webster mice; B6.129X1-*Bax*^{tm1Sjk/J} mice; Affymetrix array; Parkinson's disease

Introduction

Parkinson's disease (PD) is a progressive neurodegenerative disorder characterized by the loss of dopaminergic (DA) neurons in the substantia nigra pars compacta (SNpc). Although the etiology of idiopathic PD is unclear, it may originate from interplay between environmental agents (Landrigan et al., 2005, Brown et al., 2006) and predisposing genetic traits (Huang et al., 2004, Benmoyal-Segal and Soreq, 2006, Farrer, 2006, Wood-Kaczmar et al., 2006). However, the identification of specific genetic risk factors for idiopathic PD remains elusive.

The most widely studied experimental system of PD is the murine 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) model (Dauer and Przedborski, 2003, Smeyne and Jackson-Lewis, 2005). Although it does not recapitulate PD in its entirety, it reproduces several of its cardinal features including loss of DA neurons in the SNpc (Dauer and Przedborski, 2003, Przedborski and Vila, 2003, Smeyne and Jackson-Lewis, 2005). Moreover, as MPTP sensitivity in mice is genetically determined (Sundstrom et al., 1987, German et al., 1996, Hamre et al., 1999, Vila et al., 2001), it provides an opportunity to identify potential genetic risk factors for PD.

Several lines of evidence suggest that the primary site of injury in both PD and the MPTP model is the SNpc nerve terminals in the striatum (Bradbury et al., 1986, Herkenham et al., 1991, Nurmi et al., 2001, Rinne et al., 2001). Thus the initial event is damage of the synaptic terminals in the striatum, followed by retrograde degeneration and cell death (Bradbury et al., 1986, Eberling et al., 1997). This mechanism is particularly relevant for MPTP toxicity as its active metabolite, 1-methyl-4-phenylpyridinium (MPP⁺) selectively accumulates in DA nerve terminals in striatum via uptake through the dopamine transporter (Chiba et al., 1985, Javitch et al., 1985, Gainetdinov et al., 1997).

Shortly after its administration MPTP elicits the dumping of dopamine from SNpc nerve endings in striatum (Jackson-Lewis et al., 1995). This event coincides with the induction of several immediate-early genes (Duchemin et al., 1992, Smith et al., 1997, Perez-Otano et al., 1998, Agani et al., 2000, Chen et al., 2001). By 24 hours, depletion of striatal tyrosine hydroxylase (Kuhn et al., 2003, Sriram et al., 2004) and damage to DA synapses (Linder et al., 1995), coincide with induction of several genes, including hemeoxygenase-1 (*Hmox1*) (Fernandez-Gonzalez et al., 2000) and cytokines/chemokines (Nagatsu et al., 2000, Hebert et al., 2003, Sriram et al., 2004, Shen et al., 2005, Sriram et al., 2006, Pattarini et al., 2007). Subsequent neuronal death is progressive between 12 hours (Jackson-Lewis et al., 1995, Kuhn et al., 2003) and 7 days (Boyd et al., 2007). Based on this evidence, these early changes in gene expression could contribute to neuronal demise.

We hypothesized that sensitivity to MPTP may reside in genes expressed in striatum and their identification may point to genetic risk factors for PD.

Experimental Procedures

Animals and Experiments

Female C57BL/6J and B6.129X1-*Bax*^{tm1Sjk/J} (*Bax* +/−) mice of both genders were purchased from Jackson Laboratories (Bar Harbor, Maine, USA). *Bax* +/− mice were bred in-house and intercrossed to obtain homozygous knockout animals (*Bax* −/−) and wildtype littermates (*Bax*

+/+). The genotype for Bax mice was performed by Transnetyx (Cordova, TN, USA). Female SWR mice were purchased from Jackson Laboratories and Harlan (Indianapolis, IN, USA). Animals were housed in micro-isolator units with a 12 hour light-dark schedule and constant temperature. Further details of the Materials and Methods are provided in Pattarini et al. (2007). MPTP was administered by IP injections at the dosage and schedule specified in the Results section. Animals were sacrificed at different time points after the first dose of MPTP and brain regions were dissected and immediately frozen on dry ice to preserve RNA integrity. Samples were stored at -80°C . All studies were approved by the St. Jude Children's Research Hospital Animal Care and Use Committee (ACUC) and were conducted in accordance with the National Institute of Health Guide for the Care and Use of Laboratory Animals (NIH Publication No. 80-23, revised 1996). Efforts were made to minimize the number of animals involved in each experiment.

RNA Isolation

Total RNA was extracted with TRIzol® Reagent from Invitrogen (Carlsbad, California, USA) according to manufacturer instructions. Briefly, 1 ml TRIzol® was added to frozen samples and immediately homogenized. Samples were mixed with 200 μl of chloroform and centrifuged for 15 min at $12,000 \times g$ at 4°C . The aqueous phase was transferred to a new vial, mixed with 50 μg of glycogen from Roche Applied Science (Penzberg, Germany) and 500 μl of isopropanol and centrifuged (10 min at $12,000 \times g$, 4°C) to precipitate total RNA. The pellet was washed with ice cold 70% ethanol and air dried for 10 min. Total RNA was resuspended in RNase-free water and it was checked for integrity by agarose gel electrophoresis. Samples that appeared degraded were discarded.

Preparation of Samples for Microarray Analysis

Technical procedures for microarray analysis, including quality control of RNA, labeling, hybridization and scanning of the arrays were performed by the Hartwell Center for Bioinformatics & Biotechnology (HC) at St. Jude Children's Research Hospital (SJCRH) according to standard operating procedures for Affymetrix protocols (GeneChip® Expression Analysis manual, Affymetrix, Santa Clara CA, USA). Prior to their use, RNA sample integrity was analyzed with the 2100 Bioanalyzer Lab-on-a-chip system (Agilent Technologies, Santa Clara, CA, USA). Total RNA samples that were not degraded were labeled using the Gene Chip IVT Labeling Kit (Affymetrix) according to manufacturer instructions. Briefly, as a quality control of the labeling process, samples were first spiked with the GeneChip® Poly-A RNA Control Kit (Affymetrix) that contains mRNA (not present in eukaryotic cells) for the following *B. subtilis* genes: *lys* (1:100,000 ratio of copy number), *phe* (1:50,000), *thr* (1:25,000) and *dap* (1:7,500). Samples were then used to prepare the 1st strand cDNA using the One-Cycle cDNA Synthesis Kit (Affymetrix) containing SuperScript II followed by the 2nd strand cDNA synthesis with T4 DNA polymerase. cDNA was cleaned using cDNA Cleanup Spin Column (Affymetrix), and biotin-labeled cRNA was prepared using the Gene Chip IVT Labeling Kit (Affymetrix). Labeled cRNA was purified with Cleanup Spin Column (Affymetrix), quantified, fragmented and spiked with biotin-labeled cRNA for *bioB* (1.5 pM), *bioC* (5 pM), *bioD* (25 pM) and *Crex* (100 pM) (GeneChip® Eukaryotic Hybridization Control Kit, Affymetrix). This procedure allowed us to assess both the linearity of detection and the lowest accurately detectable concentration (1.5 pmol). Samples were loaded onto the Affymetrix® Mouse Genome 430 2.0 Arrays (Affymetrix) previously washed with 1X hybridization buffer (100 mM MES, 1M Na^+ , 20 mM EDTA, 0.01% Tween-20 pH 6.6) and hybridized overnight (16 hours) at 37°C . Arrays were washed and stained with streptavidin conjugated to phycoerythrin, using the automated GeneChip® Fluidics Station 400 (Affymetrix) and scanned to produce an image file with the GeneArray™ scanner (Affymetrix). Total RNA from each animal was loaded onto individual Affymetrix microarray chips. Experimental reproducibility

can be estimated by comparing columns within a figure as well as between corresponding columns in Figure 1, Figure 3, Figure 4, Figure 6, and Figure 8.

Analysis of Microarrays

The microarrays used in this study (Mouse Genome 430 2.0 Arrays, Affymetrix) contain 45,101 probesets, representing 39,000 transcripts and variants, and they are currently the most comprehensive genechip array available for the mouse. Scanned images were analyzed with the Gene Chip Operating Software (GCOSv1.2, Affymetrix). Assessment of probeset present/absent calls was made using the Single Array Analysis method in GCOS using the statistical algorithm with default analysis parameters (http://www.affymetrix.com/support/technical/whitepapers/sadd_whitepaper.pdf). Probeset signal values were scaled by global methods to a target value of 500.

Array analysis was performed using Spotfire® DecisionSite 8.2 from TIBCO Software Inc. (Palo alto, California, USA). The following is a brief description of the microarray data analysis procedure. First, probesets that are “Absent” across all samples were excluded (McClintick and Edenberg, 2006). The remaining probeset signals were variance-stabilized by addition of a small constant value equal to half of the average background signal (Rocke and Durbin, 2003). Variance-adjusted signals were log₂-transformed and used in the Student's t-test (two groups) or the ANOVA method (>2 groups) to identify differences in probeset expression. Probesets that satisfied the thresholds for false discovery rate < 0.05 (Benjamini and Hochberg, 1995) and fold-change (≥ 1.5 or ≤ 0.667) were selected. To identify patterns of co-regulated gene expression, the log₂-transformed signals were normalized across samples to a mean of zero and a standard deviation of one (Z-score). This procedure enables comparison of changes within the same relative magnitude. Normalized signals were analyzed by an agglomerative hierarchical clustering algorithm using the Euclidean distance and UPGMA (unweighted average) methods (Quackenbush, 2001, Butte, 2002).

Gene Set Enrichment Analysis

In addition to identifying the differentially expressed genes with an arbitrary cutoff from *t*-test followed by multiple test correction, we also compared treated samples with untreated ones at each time point using all the probesets on the array with the permutation approach. We used the R-version of a publicly available program, Gene Set Enrichment Analysis (GSEA) (Mootha et al., 2003, Subramanian et al., 2005). GSEA is a computational method that determines whether an *a priori* defined set of genes shows statistically significant, concordant differences between two biological states. We used gene sets for canonical pathways compiled by Ingenuity Pathway Analysis (<http://www.ingenuity.com>) for pathway analysis and motif gene sets from the Molecular Signature Database (Subramanian et al., 2005) for transcription factor analysis. Motif gene sets contain genes that share a cis-regulatory motif that is conserved across the human, mouse, rat and dog genomes. The motifs are catalogued in Xie *et al* (2005) and represent known or likely regulatory elements in promoters and 3'-UTRs. Only results with a value of false discovery rate ($q \leq 0.25$) were considered.

Validation of Microarray Data by Quantitative RT-PCR

Total RNA was reverse transcribed using TaqMan® reverse transcription reagents from Applied Biosystems (Foster City, California, USA). Primers and probes for real-time PCR (qRT-PCR) were designed with Primer Express Software version 1.5 (Applied Biosystems) and synthesized by the HC. Real time PCR was performed using TaqMan® PCR Core Reagent Kit (Applied Biosystem), using the ABI Prism 7900HT system (Applied Biosystem). Absolute quantification was performed using standard curves for each gene of interest. Primers and probes used for qRT-PCR are listed in Table 1.

Standards were prepared by cloning the coding sequence (CDS) of each gene into a pcDNA3 plasmid (Invitrogen) as previously described (Pattarini et al., 2007). The primers used to prepare the standards, including the restriction site used are listed in Table 2.

Statistical Analysis

Statistical methods used to analyze microarray results are explained in the microarray analysis section. Statistical analysis for qRT-PCR results was performed with GraphPad Prism® version 4.03 for Windows® (GraphPad Software Inc., San Diego, CA). Results are expressed as the ratio of number of copies of a specific gene over the number of copies of glyceraldehyde-3-phosphate dehydrogenase (*Gapdh*). Each time point is the average of at least three animals. The temporal profile of each gene was analyzed by one-way ANOVA followed by Bonferroni's multiple comparisons test to assess statistical significance versus respective control (time zero). Comparison between strains was performed either by two-way ANOVA followed by Bonferroni's multiple comparisons test (> 2 groups) or by Student's t-test (2 groups).

Results

The MPTP striatal transcriptome in C57BL/6J mice

To investigate the temporal transcriptional responses in the striatum in MPTP-sensitive strain, C57BL/6J mice were injected every two hours with either saline (as control) or MPTP (20 mg/kg) for a total of four injections. This injection schedule, sometimes referred to as the acute MPTP model, is used widely to simulate PD in mice and leads to a temporally predictable sequence of molecular and cellular events that culminate in the relatively synchronous death of SNpc neurons (Przedborski and Vila, 2003, Miller et al., 2005, Smeyne and Jackson-Lewis, 2005). Animals were sacrificed at 5 (three injections), 24 and 72 hours (both four injections) after the first dose of MPTP, the striatum removed and total RNA isolated and used for Affymetrix microarray analysis as described in Materials and Methods. These time points were chosen to span the period from the acute consequences of MPTP intoxication through times when formal perturbation of DA nerve endings and compromised SNpc neuron function are evident up to the point when DA neurons begin to die. Total RNA from each animal was loaded onto individual Affymetrix microarray chips. Experimental reproducibility can be estimated by comparing columns within a figure as well as between corresponding columns in Figure 1.

Using criteria described in the Material and Method section, we identified 107, 287 and 191 probesets as differentially expressed at 5, 24 and 72 hours, respectively (Supplemental Table 1). This target list of probesets was used to perform hierarchical cluster, Gene Ontology and Ingenuity Pathway analyses. Hierarchical cluster analysis reveals three largely discrete sets of genes whose mRNA levels change sequentially over time following MPTP administration (Figure 1 and Supplemental Table 1). At early time points (Figure 1), the levels of mRNA for a number of genes increase and then largely decline to basal values by 24 hours (Figure 1 and Supplemental Table 1). By 24 hours a distinct and larger set of mRNAs are increased (Figure 1) and then largely decline to baseline by 72 hours at which time a new set of gene expression changes are evident (Figure 1 and Supplemental Table 1). Although less in number, there were also transient decreases in some mRNAs over the same time course (Supplemental Table 1). Additional microarray data was obtained at 2 and 96 hours post-MPTP treatment. Gene expression changes seen at 2 and 96 hours were subsets of those seen at 5 and 72 hours, respectively (data not shown).

A variety of bioinformatic tools were used to analyze mRNA changes. As expected from prior studies (Duchemin et al., 1992, Doucet et al., 1996, Smith et al., 1997, Perez-Otano et al., 1998, Hunot et al., 2004), immediate early genes are prominent in the early phase following

MPTP treatment and include the transcriptional regulators early growth response 3 and 4 (*Egr3* and *Egr4*, respectively), FBJ osteosarcoma oncogene (*c-Fos*), FBJ osteosarcoma oncogene B (*Fosb*), Jun oncogene (*c-Jun*) and Jun oncogene B (*Junb*) (Supplemental Table 1). Moreover, Gene Ontology analysis revealed that transcription factors/regulators were over-represented in the early phase (20.9%) versus intermediate (5.3%) and late phases (6.3%). Similarly, Ingenuity Pathway Analysis (Ingenuity System Inc., Redwood City, CA, USA) indicated that gene expression was statistically the most over-represented function ($2.28\text{e-}08 < P < 8.72\text{e-}03$) in the early phase response. Examples include changes in mRNA levels for the transcriptional regulators BTB and CNC homology 1 (*Bach1*), B-cell translocation gene 3 (*Blg3*), CCAAT/enhancer binding protein (C/EBP), beta (*Cebpb*), Kruppel-like factor 9 (*Klf9*), nuclear receptor subfamily 4, group A, member 3 (*Nr4a3*), paired box gene 8 (*Pax8*), retinoid X receptor gamma (*Rxrg*), superoxide dismutase 2 (*Sox2*) and zinc finger and BTB domain containing 16 (*Zbtb16*).

Another major component of the early response involves genes implicated in oxidative stress and includes cyclin-dependent kinase inhibitor 1A (*Cdkn1a*), DNA-damage-inducible transcript 4 (*Ddit4*), DNA-damage-inducible transcript 4-like (*Ddit4l*), FK506 binding protein 5 (*Fkbp5*), growth arrest and DNA-damage-inducible 45 beta and gamma (*Gadd45b* and *Gadd45g*), metallothionein 2 (*Mt2*), nuclear factor of kappa light chain gene enhancer in B-cells inhibitor, alpha (*Nfkb1a*) and uncoupling protein 2 (*Ucp2*). These changes are also consistent with studies in PD and models of the disorder where evidence of oxidative stress have been reported (Kindt et al., 1987, Krueger et al., 1990, Bates et al., 1994, Hasegawa et al., 1995, Beal, 2003, Przedborski and Vila, 2003, Hald and Lotharius, 2005, Jackson-Lewis and Smeyne, 2005, Hald et al., 2007, Schapira, 2008). Other gene expression changes in the early phase represent inflammatory responses (Tnf receptor-associated factor 5 (*Traf5*) and interferon-related developmental regulator 1 (*Ifrd1*)) and steroid/stress signaling (serum/glucocorticoid regulated kinase 1 and 3 (*Sgk* and *Sgk3*), TSC22 domain family, members 1 and 3 (*Tsc22d1* and *Tsc22d3*)).

Gene set enrichment analysis (GSEA) (Mootha et al., 2003, Subramanian et al., 2005, Subramanian et al., 2007) revealed that the intermediate phase is characterized by enrichment for transcripts implicated in cytokine signaling and inflammatory responses (Supplemental Table 1 and Supplemental Table 6). This result is consistent with many studies showing the presence of inflammatory responses in striatum in both PD and animal models thereof (Hald and Lotharius, 2005, Nagatsu and Sawada, 2005, Hald et al., 2007, Hunter et al., 2007, McGeer and McGeer, 2007, Wilms et al., 2007). Expression of genes involved in TNF family signaling such as the receptors for TNF-alpha (*Tnfrsf1a*) and Tweak (*Tnfrsf12a*) are increased. Likewise, expression of genes involved in interleukin signaling pathways such as suppressor of cytokine signaling 3 (*Socs3*) and signal transducer and activator of transcription 1 and 3 (*Stat1* and *Stat3*) are elevated. Besides genes involved in cytokine and chemokine signaling, many effector molecules of the inflammatory response are increased in the intermediate phase, including the complement components 1, q subcomponent, alpha and beta polypeptide and C chain (*C1qa*, *C1qb* and *C1qc*, respectively), and complement component 4B (*C4b*); Fc receptor, IgG, high affinity I (*Fcgr1*); cathepsin B, C, D and Z (*Ctsb*, *Ctsc*, *Ctsd* and *Ctsz*, respectively); lectin, galactose binding, soluble 1 and 3 (*Lgals1* and *Lgals3*, respectively) and the *Lgals3* binding protein (*Lgals3bp*). Similarly, markers of inflammatory and immune cells such as allograft inflammatory factor 1 (*Aif1* a.k.a. *Iba1*, a microglia marker), CD antigens 44, 68, 151 and 180 (*Cd44*, *Cd68*, *Cd151* and *Cd180*, respectively), lymphocyte antigen 86 (*Ly86*), macrophage scavenger receptor 2 (*Msr2*) and oncostatin M receptor (*Osmr*) change in the intermediate phase.

Also prominent in the intermediate phase are increased transcript levels for genes associated with activation of astrocytes, including glial fibrillary acidic protein (*Gfap*) and vimentin

(*Vim*). We also, confirm our earlier demonstration of elevated *Hmox1* expression in striatal astrocytes following MPTP administration (Fernandez-Gonzalez et al., 2000). Although not a specific marker for gliosis, the levels of S100 calcium binding proteins A6, A10, A11, A13 and A16 (*S100a6*, *S100a10*, *S100a11*, *S100a13* and *S100a16*, respectively) as well as their interacting proteins, annexin A2 and A3 (*Anxa2* and *Anxa3*, respectively) (Bianchi et al., 1992, Tokumitsu et al., 1992, Rety et al., 1999, Santamaria-Kisiel et al., 2006, Rescher and Gerke, 2008) are also increased in the intermediate phase. In addition, a number of other gene products associated with protein folding, modification and elimination, such as heat shock protein 1, B6 and 8 (*Hspb1*, *Hspb6* and *Hspb8*, respectively), transglutaminase 1, K and C polypeptides (*Tgm1* and *Tgm2*, respectively) and tissue inhibitor of metalloproteinase 1 (*Timp1*) are elevated. Also indicative of ongoing responses to cellular damage and oxidative stress are elevation in levels of mRNAs for apolipoprotein D (*Apod*), fatty acid binding protein 7 (*Fabp7*) and metallothionein 2 (*Mt2*). In addition mRNA levels of genes linked with cell death such as myeloid cell leukemia sequence 1 (*Mcl1*) and transmembrane BAX inhibitor motif containing 1 (*Tmbim1*) and macroautophagy Bcl2-associated athanogene 3 (*Bag3*) (Carra et al., 2007) change in the intermediate phase. Besides gene products overtly linked to inflammation, gliosis, and cellular damage and stress responses, expression of genes involved in other signaling pathways change, including bone morphogenetic protein 1 (*Bmp1*), BMP2 inducible kinase (*Bmp2k*), CD9 antigen (*Cd9*), heparin-binding EGF-like growth factor (*Hbegf*) and transforming growth factor, beta receptor II (*Tgfb2*).

By 72 hours post-treatment the majority of the mRNA changes seen at 24 hours return to basal levels and a new cohort of transcripts are altered. The persistently altered mRNAs are those linked to gliosis, inflammation and oxidative stress and include, *Gfap*, *Vim*, *C1qc* and *C4b*, lymphocyte antigen 86 (*Ly86*), endothelin receptor type B (*Ednrb*), heat shock protein 6 (*Hspb6*), lectin, galactose binding, soluble 1 (*Lgals1*) and lectin, galactoside-binding, soluble, 3 binding protein (*Lgals3bp*), lysosomal-associated membrane protein 2 (*Lamp2*), legumain (*Lgmn*), metallothionein 1 (*Mt1*), S100 calcium binding protein A6 and A13 (*S100a6* and *S100a13*, respectively), and transferrin (*Trf*). The same inflammation/gliosis-related mRNAs are also elevated at 96 hours post treatment indicating persistent inflammatory responses and ongoing astroglial gliosis in striatum (data not shown).

In the late phase, a new cluster of gene expression changes is evident. Several immediate-early genes including early growth response 3 (*Egr3*) and Fos-like antigen 2 (*Fosl2*) are down-regulated at 72 and 96 hours. The mRNA levels for the transcription factor ets variant gene 5 (*Etv5*) and for brain-specific angiogenesis inhibitor 1-associated protein 2 (*Baiap2*), a presumptive immediate-early gene are also persistently decreased whereas levels of the transcriptional regulators activating transcription factor 6 (*Atf6*), nuclear receptor subfamily 2, group F, member 2 (*Nr2f2*) and zinc finger protein of the cerebellum 1 (*Zic1*) are increased. The mRNAs levels for many membrane and secreted proteins or proteins that modify the extracellular matrix also change at 72 hours and include aquaporin 4 (*Aqp4*), gap junction membrane channel protein alpha 1 (*Gjal* a.k.a. connexin 43), myelin oligodendrocyte glycoprotein (*Mog*), neural cell adhesion molecule 1 (*Ncam1*), proteolipid protein 1 (*Plp1*), solute carrier family 44, member 1 (*Slc44a1*), secreted acidic cysteine rich glycoprotein (*Sparc*), secreted phosphoprotein 1 (*Spp1*) and tissue inhibitor of metalloproteinase 4 (*Timp4*). Also prominent are changes in expression of genes associated with specific neuronal subtypes and include, parvalbumin (*Pvalb*), potassium voltage-gated channel, subfamily Q, member 5 (*Kcnq5*), and the gamma-aminobutyric acid (GABA) transporter solute carrier family 6, member 11, (*Slc6a11*), as well as general neuronal proteins such as bassoon (*Bsn*) and homer homolog 1 (*Homer1*). Finally, the mRNAs encoding two proteins implicated in PD, alpha-synuclein (*Snca*) (decrease) and G protein-coupled receptor 37 (*Gpr37* a.k.a. Pael-R) (increase) are altered in the late response phase. Moreover, the same changes in these two

transcripts are also evident at 96 hours suggesting that the latter two are more long-lasting alterations in gene expression (data not shown).

Assessment of temporal mRNA changes by qRT-PCR

To confirm and extend the microarray data, qRT-PCR was used to assess the temporal profiles of mRNA expression of selected genes representative of early (*Cdkn1a*, *NFkBia*, *Gadd45b*, *Fosb* and nuclear receptor subfamily 4, group A, member 1 (*Nr4a1*)) and intermediate (*Hmox1*, *Tnfrsf12a*, endothelial differentiation, sphingolipid G-protein-coupled receptor 3 (*Edg3*), PDZ and LIM domain 4 (*Pdlim4*) and *Hbegf*) phase transcripts (Figure 2). Early phase mRNAs increased between 2–5 hours post-MPTP treatment and declined to baseline by 24 hours. The only exception was *Gadd45b* that showed a small but statistically significant increase at 24 hours. The intermediate phase response transcripts increased between 12–24 hours post-MPTP treatment and declined to baseline by 3 days. These data serve to confirm and extend the microarray analysis.

Brain region specificity of MPTP-induced mRNA changes

We showed previously that *Hmox1* induction was confined to the striatum following MPTP treatment (Fernandez-Gonzalez et al., 2000). Therefore, we assessed whether expression of other genes detected in the initial microarray screen were also specifically altered in striatum. Animals were injected with MPTP (20 mg/kg) every two hours for a total of 4 injections, and sacrificed at 5 and 24 hours after the first injection and global mRNA levels in striatum, cerebral cortex and cerebellum assessed using Affymetrix microarray. Total RNA from each animal was loaded onto individual Affymetrix microarray chips. Experimental reproducibility can be estimated by comparing columns within a figure as well as between corresponding columns in Figure 3. A transient early phase of gene expression changes was evident in all three brain areas (Figure 3). However, the response was most prominent in striatum both in regards to the number of genes involved and magnitude of the changes.

In marked contrast to the early phase, expression of the intermediate phase response genes was essentially unique to the striatum (Figure 3). Moreover, there was not a different set of genes to those identified in striatum whose expression changed in cerebral cortex and cerebellum following MPTP treatment (data not shown). Therefore, there is a highly coordinated and stereotypical transcriptional response triggered by MPTP administration that is spatially and temporally restricted to the brain region that is the acute target of the neurotoxin. The visual pattern generated by the hierarchical cluster analysis program depends on the number and type of samples used for the analysis. Therefore, similar time points, may display visually different patterns in different figures although the genes within the clusters are identical.

The MPTP-induced transcriptome in the striatum of sensitive and resistant strains of mice

To establish the potential relevance of the mRNA changes observed in the striatum to the pathology elicited by MPTP we compared mRNA profiles in MPTP-sensitive (C57BL/6J) and - resistant (SWR) strains of mice. Animals of both strains were injected every two hours with either saline or MPTP 20 mg/kg for a total of 4 doses. Mice were sacrificed at 5 and 24 hours following the first injection, and striatal mRNA subjected to microarray analysis. Total RNA from each animal was loaded onto individual Affymetrix microarray chip. Total RNA from each animal was loaded onto individual Affymetrix microarray chips. Experimental reproducibility can be estimated by comparing columns within a figure as well as between corresponding columns in Figure 4. The early (5 hour) phase responses in C57BL/6J and SWR mice were indistinguishable (Figure 4). This suggests there is unlikely to be a strain-dependent difference in entry of MPTP into brain, consistent with a recent chemical determination of MPP⁺ levels in brains of C57BL/6J and SWR mice (Boyd et al., 2007). In contrast, the intermediate (24 hour) response was attenuated in SWR mice (Figure 4). Whereas the

magnitude of mRNA changes observed in C57BL/6J mice was consistent from animal to animal the degree of change in SWR mice varied greatly between animals and with respect to individual genes. Thus, some MPTP treated SWR mice were indistinguishable from saline-treated animals whereas others showed more robust responses that for some probesets approximated levels observed in sensitive C57BL/6J mice. Although SWR mice are considered MPTP resistant, this is a relative term. In the acute MPTP model, SWR mice exhibit an approximate 14 % loss of DA (tyrosine hydroxylase-positive) SNpc neurons compared with 58% loss in C57BL/6J mice under identical conditions (Hamre et al., 1999). Thus, it is expected that if the intermediate response is linked to neuronal loss, it should be evident to some extent even in SWR mice. Furthermore, the neuronal loss in SWR mice is variable, with some animals having no apparent loss whereas others have more substantial losses. Therefore, it is possible that the SWR mice with more robust intermediate responses represent animals in which cell loss would have been more substantial if they were allowed to survive, whereas those with little or no intermediate response may represent mice that would have sustained no neuronal loss.

qRT-PCR was used to quantify mRNA levels of selected early and intermediate phase genes at 2, 5 and 24 hours post-MPTP treatment in C57BL/6J and SWR mice (Figure 5). Confirming the microarray data, there were no significant inter-strain differences in mRNA levels for the 5 hour response, with all transcripts rising statistically to the same extent in both strains. In contrast, the absolute levels of transcripts for all intermediate phase response genes were lower in the striatum of the SWR strain, but the levels of attenuation varied from gene to gene. Levels of some transcripts (e.g. *Hbegf*) were not significantly altered from basal values in MPTP-treated SWR mice while others (e.g. *Hmox1*) were only slightly increased relative to saline treatment. At the other extreme, levels of some transcripts, such as *Pdlim4* were only slightly attenuated in MPTP-treated SWR mice compared to MPTP-treated C57BL/6J mice whereas others (e.g. *Edg3*) were about 50% of those observed in C57BL/6J mice (Figure 5).

These results indicate that expression of genes identified in the intermediate phase, but not the early phase are predictive of the pathological events associated with MPTP. Furthermore, some genes show more attenuation than others in the resistant strain, suggesting that they might be better candidates for being participants in the pathological response to MPTP.

Inter-strain differences in basal mRNA levels

As inter-strain differences in basal gene expression levels in striatum might contribute to MPTP sensitivity and/or the intermediate phase response we compared basal mRNA levels in striatum from SWR and C57BL/6J mice. Total RNA from each animal was loaded onto individual Affymetrix microarray chips. Experimental reproducibility can be estimated by comparing columns within a figure as well as between corresponding columns in Figure 6.

333 genes (219 higher in C57BL/6J and 114 higher in SWR) were differentially expressed between MPTP-sensitive (C57BL/6J) and MPTP-resistant (SWR) strains of mice (Figure 6 and Supplemental Table 2). The functions of the gene products involved span all GO categories, implying structural and functional differences between the striatum of the strains. Some of the transcripts (e.g. alanine-glyoxylate aminotransferase 2-like 1 (*Agxt2l1*), *Apod* and *Msr2*) are MPTP-responsive; others such as mitochondrial superoxide dismutase 2 (*Sod2*) and catechol-O-methyl transferase (*Comt*) may contribute to oxidative stress responses and dopamine metabolism, respectively. There may also be differences in microglia status between the strains as basal mRNA levels for *C1qc* and *Msr2* are markedly lower in SWR mice (Supplemental Table 2). Finally, one gene, PTEN induced putative kinase 1 (*Pink1*) has been implicated in PD (Valente et al., 2004, Bonifati et al., 2005, Tan et al., 2006, Abeliovich, 2007) and is also lower in SWR mice.

qRT-PCR was performed to measure levels of transcripts that were higher in either SWR (potassium inwardly-rectifying channel, subfamily J, member 10 (*Kcnj10*)) or C57BL/6J (*Agxt2l1*, *Apod*, *Comt* and *Pink1*) mice (Figure 7). These results confirm the microarray findings and establish that there are substantial differences in basal levels of gene expression between the two strains of mice.

The MPTP transcriptome in *Bax*^{-/-} mice

As the intermediate response is attenuated or absent in SWR mice we assessed whether MPTP-resistant *Bax*^{-/-} mice (Vila et al., 2001) show similar temporal mRNA responses to SWR mice. Moreover, as the *Bax* knockout is on an inbred C57BL/6J background we anticipate there should be fewer differences in basal gene expression between the strains. To further minimize genetic background effects we produced and analyzed both *Bax*^{-/-} and *Bax* wild type littermates by inter-crossing *Bax* heterozygous animals. These mice were treated with the standard acute MPTP paradigm and striatal mRNA levels analyzed by Affymetrix and qRT-PCR at 24 hours post-treatment. Total RNA from each animal was loaded onto individual Affymetrix microarray chips. Experimental reproducibility can be estimated by comparing columns within a figure as well as between corresponding columns in Figure 8.

There are fewer (59 genes) differences in basal mRNA expression levels between *Bax*^{-/-} and *Bax* wild type mice (Supplemental Table 3). Besides the expected loss of *Bax* mRNA, there was also loss of GABA-A receptor, subunit gamma 3 (*Gabrg3*) and the small nuclear ribonucleoprotein *Snurf*. As both genes lie close to *Bax* on chromosome 7 it is possible that the homologous recombination event that generated the *Bax*^{-/-} allele has affected the structure and/or expression of neighboring genes. Of the differentially expressed genes, only the elevated levels of huntingtin-associated protein 1 (*Hap1*) mRNA in *Bax*^{-/-} mice has overt implications for neurodegeneration.

Unlike SWR mice there was a robust intermediate response in *Bax*^{-/-} mice that was qualitatively (number and identity of genes) and quantitatively (levels of mRNA) largely indistinguishable from that seen in wild type littermates (Figure 8 and Supplemental Table 4). Using qRT-PCR for selected intermediate response genes, all tested transcripts in *Bax*^{-/-} mice increased to at least the same levels observed in *Bax* wild type littermates (Figure 9). In fact, levels of *Tnfrsf12a* mRNA increased to a significantly higher level in *Bax*^{-/-} mice compared to wild type mice.

Discussion

We showed previously that acute intoxication of DAergic synapses in the striatum with MPTP induces Hmox1 in surrounding astrocytes (Fernandez-Gonzalez et al., 2000). Based upon these data we proposed that products of Hmox1, such as carbon monoxide and iron, constituted a feed-forward loop that could further damage nerve terminals leading to neuronal death (Fernandez-Gonzalez et al., 2000, Smeyne and Jackson-Lewis, 2005). Here we have expanded this hypothesis using a genome-wide approach to show that Hmox1 is but one representative of a large cohort of genes that undergo stereotypical temporal and spatial patterns of change in the MPTP model. We therefore suggest a scenario in which the initial damage to the DA nerve endings in the striatum elicited by MPTP, initiates a second wave of gene expression events in surrounding cells whose products provide the final *coup de grace* to the DA neurons. Genetic resistance to MPTP can therefore take at least two forms. In SWR mice, the coupling between the initial damage and the secondary response is disrupted. In *Bax*^{-/-} mice, however, resistance is conferred by an ability of the neurons to resist both the primary and secondary insults.

The present data establish that there are stereotypical changes in striatal mRNA levels following MPTP administration that reflect a number of biological and pathological responses triggered by MPTP treatment. Whereas the transient acute changes in mRNA levels elicited by MPTP are not specific to striatum and are evident in both sensitive and resistant strains of mice, the intermediate and late mRNA responses are confined to the striatum and are coincident with the period in which dopaminergic synaptic terminals in this brain region undergo functional and structural damage and neuronal death is initiated. As such the delayed phases are predictive of MPTP sensitivity, and may contribute, or be responsive to the pathogenic mechanism of MPTP. The fact that the early phase in striatum is qualitatively (genes represented) and quantitatively (mRNA levels) indistinguishable between C57BL/6J and SWR mice suggests that peripheral metabolism and/or penetration of MPTP into the brain is not the underlying mechanism of resistance in the latter strain. Rather the data are consistent with a resistance mechanism in SWR involving limitation of nerve terminal damage and/or attenuation of the intermediate transcriptional response. In contrast, in *Bax*^{-/-} mice there is a robust intermediate response implying a different mode of MPTP resistance where damage to DA synaptic terminals, inflammation and astrogliosis in the striatum is tolerated by the DAergic SNpc neurons.

The early response has two general features. First, a subset of genes is induced to similar levels in all brain regions examined. Most of these genes have been implicated in responses to oxidative stress and include *Cdkn1a* (Hershenson, 2004, O'Reilly, 2005), *Gadd45g* (Edwards et al., 2004) and FK506 binding protein 5 (*Fkbp5*) (Grigoryev et al., 2006) implying widespread metabolic compromise, presumptively attributable to inhibition of mitochondrial respiration by MPP⁺ (Kindt et al., 1987, Krueger et al., 1990, Bates et al., 1994, Hasegawa et al., 1995). These findings are consistent with an extensive literature showing oxidative stress and cytokine signaling responses in the MPTP model (Beal, 2003, Przedborski and Vila, 2003, Hald and Lotharius, 2005, Jackson-Lewis and Smeyne, 2005, Hald et al., 2007, Schapira, 2008). Second there is a striatum enriched component of the early response involving a larger number of genes whose levels of expression change in the order striatum > cerebral cortex » cerebellum. For example, many immediate-early genes are induced to lesser extents (e.g. *Fosb*, *Junb*) or not at all (e.g. *c-Jun*) in cortex and cerebellum when compared to striatum. A number of additional genes linked to oxidative stress responses such as metallothionein 2 (*Mt2*), uncoupling protein 2 (*Ucp2*), *Gadd45b* and *NFkBia* are also increased to greater extents in striatum, suggesting that this structure may experience additional metabolic stress. MPP⁺ preferentially accumulates in dopaminergic nerve terminals and elicits dumping of DA (Chiba et al., 1985, Rollema et al., 1988), a potent oxidant as well as a receptor ligand. Therefore, the striatum selective responses may be the result of dopamine receptor activation and local oxidative damage caused by dopamine. The finding of elevated *Fosb* in striatal neurons in the MPTP model (Perez-Otano et al., 1998) is consistent with dopamine receptor interactions mediating some of the early responses. Furthermore, the non-receptor-mediated induction of *Hmox1* by dopamine (but not other catecholamines) in glia (Schmidt et al., 1999) also supports a local oxidative stress model.

Some of the early mRNA changes may also be triggered as part of the central stress response to the peripheral toxicity of MPTP. Indeed many stressors trigger expression of immediate-early genes in the brain (Morgan and Curran, 1989, Schreiber et al., 1991, Liu et al., 2001, de Kloet et al., 2005, Grottick et al., 2005). Moreover, several genes identified in the early response are known to be responsive to stress/steroid hormone signaling, including Serum/glucocorticoid regulated kinases 1 and 3 (*Sgk*, and *Sgk3*, respectively) and TSC22 domain family, members 1 and 3 (*Tsc22d1* and *Tsc22d3*, respectively) (Leung et al., 2003) and some have been implicated in the MPTP model of PD (Schoenebeck et al., 2005, Stichel et al., 2005). Similarly, DNA-damage-inducible transcript 4 (*Ddit4*) which is implicated in the mTOR-signaling pathway and whose levels are increased in Parkinson brain (Malagelada et al., 2006) and in a 6OHDA cellular model of PD (Malagelada et al., 2006) increases in the

early phase. Interestingly, we observed not only an increase of DNA-damage-inducible transcript 4 (*Ddit4*) mRNA levels, but also a decrease in mRNA for the related gene, DNA-damage-inducible transcript 4-like (*Ddit4l*) in the same time period (Supplementary Table 1). As these changes occur in both sensitive and resistant strains of mice, their relevance to the pathology of PD is questionable. Nonetheless, in order to understand their relevance, it will be important to ascertain whether these reciprocal changes in gene expression occur in the same cell population.

The intermediate phase is characterized by the over-representation of transcripts implicated in cytokine signaling, inflammatory responses, activation of astrocytes and responses to cellular stress and damage (Supplemental Table 1 and Supplemental Table 6). Many studies have reported the presence of inflammatory responses in striatum in both PD and animal models of the disease (Hald and Lotharius, 2005, Nagatsu and Sawada, 2005, Hald et al., 2007, Hunter et al., 2007, McGeer and McGeer, 2007, Wilms et al., 2007) as well as astrogliosis and glial activation (McGeer et al., 1988, Francis et al., 1995, Kohutnicka et al., 1998, McGeer and McGeer, 2007). Besides gene products overtly linked to inflammation, gliosis, and cellular damage and stress responses, a number of other signaling pathways are also apparently altered during the intermediate response to MPTP. For example, levels of both CD9 antigen (*Cd9*) and *Hbegf* increase. As CD9 antigen associates with and activates the membrane bound form of heparin-binding EGF (Iwamoto et al., 1994) this coordinate up-regulation is expected to increase signaling through this pathway. There may also be changes in the bone morphogenetic protein and transforming growth factors signaling pathways following MPTP treatment as there are alterations in the mRNA levels of bone morphogenetic protein 1 (*Bmp1*) and BMP2 inducible kinase (*Bmp2k*) as well as the transforming growth factor, beta receptor II (*Tgfb2*).

With a few notable exceptions, by 72 hours post-treatment the majority of the mRNA changes seen at 24 hours return to basal levels and a new cohort of transcripts are altered. Prominent, amongst the persistently altered mRNAs are those linked to gliosis, inflammation and oxidative stress. As these same gene expression changes are evident at 96 hours, this provides evidence for an ongoing and persistent inflammatory response in striatum that initiates within 24 hours of MPTP treatment. Nevertheless, the majority of genes whose expression is altered at 24 hours have returned to basal levels and another cohort of mRNA changes is evident at 72 and 96 hours.

The biological functions of genes that alter uniquely in the late phase are diverse and potentially represent adaptive responses occurring in neurons and oligodendrocytes as well as astrocytes and microglia. For example, changes in parvalbumin and solute carrier family 6, member 11 (*Slc6a11*, a GABA transporter) expression imply alterations in striatal GABAergic interneurons (Kawaguchi et al., 1995, Marin et al., 2000) whereas changes in myelin oligodendrocyte glycoprotein (*Mog*), 2',3'-cyclic nucleotide 3' phosphodiesterase (*Cnp*) and proteolipid protein 1 (*Plp1*) indicate responses in oligodendrocytes (Scolding et al., 1989, Hardy and Friedrich, 1996, Jakovcevski and Zecevic, 2005). A number of mRNAs in the late phase encode membrane or secreted proteins involved in intercellular communication and extracellular matrix function such as neural cell adhesion molecule 1 (*Ncam1*), gap junction membrane channel protein alpha 1 (*Gjal* a.k.a. connexin 43), secreted acidic cysteine rich glycoprotein (*Sparc*), secreted phosphoprotein 1 (*Spp1*) and tissue inhibitor of metalloproteinase 4 (*Timp4*). These responses may reflect the process of synaptic terminal elimination and remodeling as might changes in mRNA levels for the synaptic protein, bassoon (*Bsn*).

The mRNA levels of a number of genes identified in this analysis have been reported to change in various MPTP models. However, as the models diverge in terms of dosing regimens, brain regions studied, time courses examined as well as microarray/analytical platforms, statistical

criteria and sample size used, we cannot readily make direct comparisons, although we can highlight similarities among the present analysis and previously published reports. Here we identify 443 genes of which 88 belong to the early response (68 increased and 20 reduced), 226 to the intermediate response (199 increased and 27 reduced) and 170 to the late response (108 increased and 62 reduced). Moreover, we have chosen time points when cell death in the SNpc has not yet started (early response), is in its infancy (intermediate response) or is advanced (late response). When we compared previously published results obtained in striatum of MPTP-treated mice with our dataset we observed relatively little overlap. Using cDNA microarray techniques, Grünblatt and colleagues identified 51 genes whose levels were modulated 8 days after the first MPTP treatment in the striatum (Grünblatt et al., 2001, Mandel et al., 2002), only one of which, solute carrier family 6, member 11 (*Slc6a11*), was detected in our analysis (elevated at 3 days post treatment). The analysis of the striatal response to MPTP performed by Miller and colleagues using Affymetrix arrays (U74A v2) revealed 178 and 716 genes modulated at 8 and 15 days, respectively, after the first MPTP injection (Miller et al., 2005). Of these genes, only 25 were modulated in a similar fashion in our study (early response Kit ligand (*Kitl*), metallothionein 2 (*Mt2*) and serine/arginine-rich protein specific kinase 1 (*Srpkl1*); intermediate response AMP deaminase 3 (*Ampd3*), cytochrome b-245, alpha polypeptide (*Cyba*), *C1qb*, metallothionein 2 (*Mt2*) and oncostatin M receptor (*Osmr*); late response angiotensinogen (*Agt*), cathepsin S (*Ctss*), human immunodeficiency virus type I enhancer binding protein 2 (*Hivep2*), myelin oligodendrocyte glycoprotein (*Mog*), ribonuclease T2A and B (*Rnaset2a* and *Rnaset2b*), Rho-associated coiled-coil containing protein kinase 2 (*Rock2*), secreted phosphoprotein 1 (*Spp1*) and tropomyosin 1, alpha (*Tpm1*) and intermediate/late *Apod*, aquaporin 4 (*Aqp4*), *C1qc*, *Gfap*, lectin, galactose binding, soluble 1 (*Lgals1*), metallothionein 1 (*Mt1*), S100 calcium binding proteins A6 and A13 (*S100a6* and *S100a13*, respectively) and TYRO protein tyrosine kinase binding protein (*Tyrobp*)). In a recent study (Chin et al., 2008) using a similar dosing paradigm (15 mg/kg every 2 hours, 4 injections) and the same Affymetrix chip used here, Chin and coworkers identified 181 genes whose mRNA are changed in the striatum of C57BL/6J mice 7 days after MPTP treatment. Ten genes identified in the latter study were also detected in our analysis: 8 genes were elevated 7 days post MPTP treatment and were similarly regulated in the intermediate and late responses in our study (oncostatin M receptor (*Osmr*) and *Serpina3n*, both elevated at 24 hours; and ATP-binding cassette, sub-family A (ABC1), member 1 (*Abca1*), aquaporin 4 (*Aqp4*), *Gfap*, lymphocyte antigen 86 (*Ly86*), transcription factor 7-like 2, T-cell specific, HMG-box (*Tcf7l2*) and *Vim* elevated at both 24 and 72 hours). The remaining two genes in common were downregulated (protein tyrosine phosphatase 4a3 (*Ptp4a3*) and ryanodine receptor 1 (*Ryr1*), both decreased at 72 hours). Despite the relatively low overlap, the genes consistently identified by all studies suggests long-term changes in processes such as inflammation, astrogliosis and protein trafficking. Our analysis indicates that these processes are initiated within the first 24 hours of treatment. Therefore, strategies aimed at ameliorating damage likely need to target early events that couple the insult to the pathological responses.

The early response to MPTP treatment in striatum involves changes in expression levels of many genes implicated in transcriptional regulation (approximately 21%) and is replete in immediate-early gene transcription factors such as early growth response 3 and 4 (*Egr3* and *Egr4*, respectively), *Fos*, *Fosb*, *Jun* and *Junb* (Supplemental Table 1). In addition to transient increases in immediate-early gene expression there are increases in transcriptional repressors such as *Bach1* (Oyake et al., 1996), B-cell translocation gene 3 (*Btg3*) (Ou et al., 2007) and zinc finger and BTB domain containing 16 (*Zbtb16*) (Costoya, 2007) as well as putative activators such as Kruppel-like factor 9 (*Klf9*) (Zhang et al., 2003) and transient decreases in others such as retinoid X receptor gamma (*Rxrg*) and paired box gene 8 (*Pax8*). The implication is that these alterations in turn trigger subsequent changes in expression of other target genes, such as those in the intermediate response. Whereas the acute response occurs to the same extent in both sensitive and resistant strains, the intermediate response is much attenuated in

SWR mice, suggesting that the first is not causative of the second. However, we cannot exclude the possibility that the early responses are essential but not sufficient to trigger the intermediate phase response. In this scenario the mechanism of resistance in the SWR strain could involve the uncoupling of the early transcriptional response from the intermediate response. The late response is also characterized by changes in expression of several transcription factors. Notably, the immediate-early gene transcription factors, early growth response 3 (*Egr3*) and Fos-like antigen 2 (*Fosl2*) that are up-regulated in the early response are actually down-regulated in the late phase. This is reminiscent of the behavior of *c-Fos* following seizures, where its levels first increase and then decline to below basal values, at which point the gene becomes un-responsive or refractory to re-induction by subsequent challenges with chemoconvulsants (Morgan et al., 1987). Levels of mRNA for the transcription factor, ets variant gene 5 (*Etv5*) are also decreased in the late phase whereas levels of the transcriptional regulators activating transcription factor 6 (*Atf6*), nuclear receptor subfamily 2, group F, member 2 (*Nr2f2*) and zinc finger protein of the cerebellum 1 (*Zic1*) are increased. This again implies that MPTP elicits coordinated transcriptional cascades in striatum that are correlated with pathology. Like MPTP, methamphetamine also causes damage to DAergic synapses in striatum (O'Callaghan and Miller, 1994). Using a cDNA array platform Cadet et al. (2001) showed that methamphetamine treatment elicited a rapid (2–4 hours) increase in the levels of many mRNAs. As in the MPTP model, this early component was enriched in transcripts encoding transcription factors/DNA binding proteins (33.6% and 35.4%, respectively) many of which were immediate-early genes. Although a direct comparison is limited by the differences in platform and strains of mice (CD1) used, several genes including c-Jun, c-Fos, Pax8, JunB and FosB are in common with our dataset. This suggests the early component may be part of a common striatal response to synaptic impairment/damage. The same study also reported gene expression changes at 16 hours post-treatment, a time intermediate between the 5 and 24-hour time points investigated here. Comparing their 16 hour dataset with our 24 hour dataset revealed only three gene products, Cathepsin D, GADD45 and Stat3 to be in common. However, the time differences between the studies do not enable us to conclude whether or not methamphetamine elicits the same intermediate response as MPTP.

Although we determined the temporal relationships of gene expression changes in striatum in response to MPTP our methods do not have cellular resolution thereby limiting interpretation of signaling cascades. i.e. we cannot prove that any two changes in gene expression occur in the same cell. Nevertheless, valuable information can be mined from the data regarding potential signaling pathways activated by MPTP. To identify transcriptional regulators in the early response that potentially contribute to changes in the intermediate response, we used the Molecular Signature Database (MSigDB, www.broad.mit.edu/gsea/msigdb/msigdb_index.html) (Supplemental Table 8, Supplemental Table 9 and Supplemental Table 10). The most significant transcription factor binding site associations to genes during the intermediate response include JunD, Nrf2, Stat1 and Stat3, Bach1 and Bach2, and members of the NFkB, AP-1 and E2F families. Levels of mRNAs for both STATs increased in the intermediate response and presumptively contribute to cytokine signaling associated with the inflammatory response. Levels of *Bach1* mRNA increase in the early phase. Bach signaling has been implicated in regulation of *Cdkn1a* expression (Shim et al., 2006) that is ubiquitously and acutely up regulated by MPTP. In addition, putative Bach1 binding sites are present in several genes identified in the intermediate response, including *Gfap*, S100 calcium binding protein A10 (*S100a10*) and *Tnfrsf12a*. Moreover, induction of *Hmox1* requires NRF2 to inactivate the transcriptional repressor Bach1 (Reichard et al., 2007). Putative Elf1 binding sites are also enriched in the intermediate phase. Elf1 is an Ets-related transcription factor that can associate with other transcription factors, such as AP-1 complexes (Bassuk and Leiden, 1995) and has been implicated in gene regulation in many contexts, most notably immune and inflammatory responses (Serdobova et al., 1997, Gallant and Gilkeson, 2006). Putative Elf-1 sites are present in genes encoding *C1qa*, Fc receptor, IgE,

high affinity I, gamma polypeptide (*Fcer1g*) and *Vim* that increase in the intermediate response. Moreover, *Vim* also harbors an AP-1 site, members of which increase in both the early (e.g. *Fos*, *Junb*) and intermediate (*c-Jun*) responses opening the possibility for co-regulation through Elf1-AP-1 associations. An Elf1 site is also present in allograft inflammatory factor 1 (*Aif1* a.k.a. *Iba1*) a marker for microglia (Ito et al., 1998) whose expression also increases in the intermediate response. Thus, Elf1 may contribute to both microgliosis and astrogliosis in the MPTP model.

The relative resistance to MPTP in SWR versus C57BL/6J mice is a polygenic trait (Cook et al., 2003) that could be attributable to differences in both the basal and MPTP modulated levels of gene expression as well as amino acid polymorphisms. In striatum alone 333 genes are differentially expressed between the strains (Supplemental Table 2) and several could potentially contribute to MPTP resistance. For example, superoxide dismutase 2 (*Sod2*) has been implicated in oxidative stress responses and *Comt* contributes to dopamine metabolism. Of the 333 transcripts, 12 (e.g. *Agxt2l1*, *ApoD*, *Msr2*) are also regulated by MPTP and belong almost exclusively to the intermediate and late phases that are attenuated in SWR mice (Supplemental Table 11). Notably, a number of the regulated genes, such as *Clqc* and *Msr2* are likely expressed in microglia and are reduced in abundance in SWR mice even under basal conditions. Furthermore, another gene with reduced expression in SWR mice, CD34 antigen (*Cd34*) has been associated with microgliosis (Ladeby et al., 2005) whereas the complement antagonist *Cd59a* (Qian et al., 2000) that attenuates damage in experimental allergic encephalitis (Mead et al., 2004) is elevated in SWR mice. This could imply intrinsic functional differences in microglia between the strains that warrant further analysis in the MPTP model.

A previous quantitative trait loci (QTL) analysis identified a region of chromosome 1 (*mptp1*) that showed a significant association with the strain dependent differences in MPTP sensitivity in SWR and C57BL/6J mice (Cook et al., 2003). We identified 3 genes (*Kcnj10*, nuclear VCP-like (*Nvl*) and signal recognition particle 9 (*Srp9*)) in the *mptp1* locus that are differentially expressed between the strains (Supplemental Table 12 and Figure 9). In addition, mRNA levels for another 6 genes in the locus alter following MPTP treatment in C57BL/6J mice (Supplemental Table 13). These genes therefore become candidates for more detailed analysis.

The results in SWR mice indicate that both the inflammatory response and gliosis seen in C57BL/6J mice is attenuated in the resistant strain. However, this is not a universal mechanism of MPTP resistance as *Bax*^{-/-} mice exhibit a robust intermediate response that is qualitatively and quantitatively indistinguishable from wild type littermates. In addition, *Bax*^{-/-} mice have very few intrinsic differences in their basal striatal mRNA profiles compared to wild type littermates. Of the differentially expressed transcripts, only the elevated levels of huntingtin-associated protein 1 (*Hap1*) mRNA in *Bax*^{-/-} mice has any overt implications for neurodegeneration. Huntingtin-associated protein 1 (*Hap1*) can bind and sequester polyglutamine-expanded proteins such as Huntingtin thereby antagonizing aggregate formation (Li et al., 1995). Therefore, it is conceivable that this leads to greater MPTP resistance in *Bax*^{-/-} mice. An alternative hypothesis is that *Bax* resistance lies downstream of the inflammatory response, possibly in the SNpc DA neurons themselves, making them tolerant of the insult. These possibilities could be tested using a floxed *Bax* allele and a Cre recombinase targeted to dopaminergic neurons.

The present data provide insight into the biological and pathological processes triggered by MPTP treatment as well as the genes and mechanisms that might contribute to sensitivity to this neurotoxin. However, the ultimate goal of the study is to identify genetic factors that contribute to PD. Analysis of our datasets for genes that have been linked to PD, identified 3 candidates: *Gpr37* (a.k.a. *PaelR*) and alpha-synuclein (*Snca*) that are increased and decreased,

respectively, at 72 hours post-MPTP treatment, and *Pink1* that is expressed at higher levels in the MPTP-sensitive C57BL/6J strain of mice.

Polymorphism of *Snca* have been linked with familial (Polymeropoulos et al., 1997, Kruger et al., 1998) and sporadic forms of PD (Chiba-Falek et al., 2006, Ahn et al., 2008). Although the role of *Snca* in PD has been extensively reviewed (Dauer and Przedborski, 2003, Cookson, 2005, Schapira, 2006, Wood-Kaczmar et al., 2006), its physiological and pathophysiological roles remain elusive. Several functions have been ascribed to this protein such as: activation of microglia (Su et al., 2007), modulation of glutamatergic (Gureviciene et al., 2007) and DAergic release (Abeliovich et al., 2000) and regulation of NFkB signaling pathway (Yuan et al., 2007). The orphan G-protein coupled receptor, *Gpr37* (Marazziti et al., 1997, Marazziti et al., 1998) is a known substrate for Parkin (Imai et al., 2003, Imai et al., 2007), another gene linked to familial forms of PD (Kitada et al., 1998). *Gpr37* has been implicated in DA metabolism (Imai et al., 2007) as well as in the death of DA neurons in the SNpc (Kitao et al., 2007). *Pink1* (PTEN-inducible kinase 1) is a serine/threonine protein kinase (Unoki and Nakamura, 2001) localized in the cytoplasm (Haque et al., 2008) and mitochondria (Valente et al., 2004). Mutations in *Pink1* segregate with familial forms of PD (Valente et al., 2004, Albanese et al., 2005, Bonifati et al., 2005, Cookson, 2005). *Pink1* has been reported to protect cells from apoptosis (Petit et al., 2005) and neurons from MPTP toxicity (Haque et al., 2008). Although its genetic elimination does not cause DA neuron loss (Zhou et al., 2007), *Pink1* knockout mice display altered striatal DA release and synaptic plasticity (Kitada et al., 2007). Its lower levels in SWR mice seems at odds with the suggested pro-survival effect of *Pink1* in PD (Abeliovich, 2007, Pridgeon et al., 2007).

A major question raised by this study is to what extent the risk for, and/or progression of PD may be influenced by the transcriptome of the striatum versus that of the SNpc. This issue is especially relevant as a number of gene products linked to PD are broadly expressed in the brain and it is sometimes unclear how they result in the specific pattern of neurodegeneration seen in this disease. For example, *Pink1* is widely expressed in brain (Taymans et al., 2006) and how disruption of its function leads to PD is unclear. Hence changed *Pink1* expression or function in striatum might be critical in PD. Alpha-synuclein and the Parkin substrate, *Gpr37* are also broadly expressed in brain (Jakes et al., 1994, Zeng et al., 1997, Li et al., 2002, Kingsbury et al., 2004) as is Parkin itself (Stichel et al., 2000). However, *Gpr37* and *Snca* are components of Lewy bodies (Arima et al., 1999, Ishizawa et al., 2003, Murakami et al., 2004, Wakabayashi et al., 2007) and it is most likely that their pathophysiological roles are exerted in SNpc DAergic neurons. To resolve the question of the pathophysiological relevant sites of expression of susceptibility genes for PD it will be necessary to selectively alter levels of their transcripts in either SNpc or striatum by genetic means.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Abbreviations

Agxt2l1, alanine-glyoxylate aminotransferase 2-like 1
 Apod, apolipoprotein D
 Bach1, BTB and CNC homology 1
 Bax, BCL2-associated X protein
 C1qa, complement component 1, q subcomponent, alpha polypeptide
 C1qb, complement component 1, q subcomponent, beta polypeptide
 C1qc, complement component 1, q subcomponent, C chain
 C4b, complement component 4B (Child blood group)

Cdkn1a, cyclin-dependent kinase inhibitor 1A
 c-Fos, FBJ osteosarcoma oncogene
 c-Jun, Jun oncogene
 Comt, catechol-O-methyl transferase
 DA, dopamine
 Edg3, endothelial differentiation, sphingolipid G-protein-coupled receptor, 3
 Fosb, FBJ osteosarcoma oncogene B
 GABA, gamma-aminobutyric acid
 Gadd45b, Growth arrest and DNA-damage-inducible 45 beta
 Gfap, Glial fibrillary acidic protein
 Gpr37, G protein-coupled receptor 37
 GSEA, gene set enrichment analysis
 Hbegf, heparin-binding EGF-like growth factor
 Hmox1, hemeoxygenase-1
 Junb, Jun-B oncogene
 Kcnq10, potassium voltage-gated channel, subfamily Q, member 10
 MPTP, 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine
 MPP⁺, 1-methyl-4-phenylpyridinium
 Msr2, macrophage scavenger receptor 2
 Nfkb1a, Nuclear factor of kappa light chain gene enhancer in B-cells inhibitor, alpha
 PD, Parkinson's disease
 Pdlim4, PDZ and LIM domain 4
 Pink1, PTEN induced putative kinase 1
 qRT-PCR, quantitative reverse transcription polymerase chain reaction
 Snca, synuclein, alpha
 SNpc, substantia nigra pars compacta
 Tnfrsf12a, tumor necrosis factor receptor superfamily, member 12a
 Vim, vimentin

Acknowledgement

The authors would like to thank Dr. D. Solecki for critical reading of this manuscript. We also thank the Hartwell Center for Bioinformatics and Biotechnology for the synthesis of realtime PCR primers and probes, sequencing and for processing samples for Affymetrix GeneChip technology. This work was supported in part by the National Institutes of Health Cancer Center CORE Grant CA 21765, the American Lebanese Syrian Associated Charities (ALSAC) and the National Institutes of Health grants R01-NS042828 and R01-ES010772 to J.I.M.

References

- Abeliovich A. Parkinson's disease: pro-survival effects of PINK1. *Nature* 2007;448:759–760. [PubMed: 17700685]
- Abeliovich A, Schmitz Y, Farinas I, Choi-Lundberg D, Ho WH, Castillo PE, Shinsky N, Verdugo JM, Armanini M, Ryan A, Hynes M, Phillips H, Sulzer D, Rosenthal A. Mice lacking alpha-synuclein display functional deficits in the nigrostriatal dopamine system. *Neuron* 2000;25:239–252. [PubMed: 10707987]
- Agani FH, Pichiule P, Chavez JC, LaManna JC. The role of mitochondria in the regulation of hypoxia-inducible factor 1 expression during hypoxia. *The Journal of biological chemistry* 2000;275:35863–35867. [PubMed: 10961998]
- Ahn TB, Kim SY, Kim JY, Park SS, Lee DS, Min HJ, Kim YK, Kim SE, Kim JM, Kim HJ, Cho J, Jeon BS. alpha-Synuclein gene duplication is present in sporadic Parkinson disease. *Neurology* 2008;70:43–49. [PubMed: 17625105]
- Albanese A, Valente EM, Romito LM, Bellacchio E, Elia AE, Dallapiccola B. The PINK1 phenotype can be indistinguishable from idiopathic Parkinson disease. *Neurology* 2005;64:1958–1960. [PubMed: 15955954]

- Arima K, Hirai S, Sunohara N, Aoto K, Izumiyama Y, Ueda K, Ikeda K, Kawai M. Cellular co-localization of phosphorylated tau- and NACP/alpha-synuclein-epitopes in lewy bodies in sporadic Parkinson's disease and in dementia with Lewy bodies. *Brain research* 1999;843:53–61. [PubMed: 10528110]
- Bassuk AG, Leiden JM. A direct physical association between ETS and AP-1 transcription factors in normal human T cells. *Immunity* 1995;3:223–237. [PubMed: 7648395]
- Bates TE, Heales SJ, Davies SE, Boakye P, Clark JB. Effects of 1-methyl-4-phenylpyridinium on isolated rat brain mitochondria: evidence for a primary involvement of energy depletion. *Journal of neurochemistry* 1994;63:640–648. [PubMed: 8035188]
- Beal MF. Mitochondria, oxidative damage, and inflammation in Parkinson's disease. *Ann N Y Acad Sci* 2003;991:120–131. [PubMed: 12846981]
- Benjamini Y, Hochberg Y. Controlling the false discovery rate: a practical and powerful approach to multiple testing. *Journal of the Royal Statistical Society* 1995;57:289–300.
- Benmoyal-Segal L, Soreq H. Gene-environment interactions in sporadic Parkinson's disease. *Journal of neurochemistry* 2006;97:1740–1755. [PubMed: 16805780]
- Bianchi R, Pula G, Ceccarelli P, Giambanco I, Donato R. S-100 protein binds to annexin II and p11, the heavy and light chains of calpactin I. *Biochimica et biophysica acta* 1992;1160:67–75. [PubMed: 1420334]
- Bonifati V, Rohe CF, Breedveld GJ, Fabrizio E, De Mari M, Tassorelli C, Tavella A, Marconi R, Nicholl DJ, Chien HF, Fincati E, Abbruzzese G, Marini P, De Gaetano A, Horstink MW, Maat-Kievit JA, Sampaio C, Antonini A, Stocchi F, Montagna P, Toni V, Guidi M, Dalla Libera A, Tinazzi M, De Pandis F, Fabbrini G, Goldwurm S, de Klein A, Barbosa E, Lopiano L, Martignoni E, Lamberti P, Vanacore N, Meco G, Oostra BA. Early-onset parkinsonism associated with PINK1 mutations: frequency, genotypes, and phenotypes. *Neurology* 2005;65:87–95. [PubMed: 16009891]
- Boyd JD, Jang H, Shepherd KR, Faherty C, Slack S, Jiao Y, Smeyne RJ. Response to 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) differs in mouse strains and reveals a divergence in JNK signaling and COX-2 induction prior to loss of neurons in the substantia nigra pars compacta. *Brain research* 2007;1175:107–116. [PubMed: 17884023]
- Bradbury AJ, Costall B, Jenner PG, Kelly ME, Marsden CD, Naylor RJ. MPP+ can disrupt the nigrostriatal dopamine system by acting in the terminal area. *Neuropharmacology* 1986;25:939–941. [PubMed: 3774119]
- Brown TP, Rumsby PC, Capleton AC, Rushton L, Levy LS. Pesticides and Parkinson's disease--is there a link? *Environmental health perspectives* 2006;114:156–164. [PubMed: 16451848]
- Butte A. The use and analysis of microarray data. *Nat Rev Drug Discov* 2002;1:951–960. [PubMed: 12461517]
- Cadet JL, Jayanthi S, McCoy MT, Vawter M, Ladenheim B. Temporal profiling of methamphetamine-induced changes in gene expression in the mouse brain: evidence from cDNA array. *Synapse* 2001;41:40–48. [PubMed: 11354012]
- Carra S, Seguin SJ, Lambert H, Landry J. HSPB8 chaperone activity towards poly-Q containing proteins depends on its association with BAG3, a stimulator of macroautophagy. *The Journal of biological chemistry*. 2007
- Chen JY, Hsu PC, Hsu IL, Yeh GC. Sequential up-regulation of the c-fos, c-jun and bax genes in the cortex, striatum and cerebellum induced by a single injection of a low dose of 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) in C57BL/6 mice. *Neuroscience letters* 2001;314:49–52. [PubMed: 11698144]
- Chiba-Falek O, Lopez GJ, Nussbaum RL. Levels of alpha-synuclein mRNA in sporadic Parkinson disease patients. *Mov Disord* 2006;21:1703–1708. [PubMed: 16795004]
- Chiba K, Trevor AJ, Castagnoli N Jr. Active uptake of MPP+, a metabolite of MPTP, by brain synaptosomes. *Biochem Biophys Res Commun* 1985;128:1228–1232. [PubMed: 3873939]
- Chin MH, Qian WJ, Wang H, Petyuk VA, Bloom JS, Sforza DM, Lacan G, Liu D, Khan AH, Cantor RM, Bigelow DJ, Melega WP, Camp DG 2nd, Smith RD, Smith DJ. Mitochondrial Dysfunction, Oxidative Stress, and Apoptosis Revealed by Proteomic and Transcriptomic Analyses of the Striata in Two Mouse Models of Parkinson's Disease. *J Proteome Res*. 2008Epub ahead of print (PMID: 18173235)

- Cook R, Lu L, Gu J, Williams RW, Smeyne RJ. Identification of a single QTL, Mptp1, for susceptibility to MPTP-induced substantia nigra pars compacta neuron loss in mice. *Brain Res Brain Res Rev* 2003;110:279–288.
- Cookson MR. The biochemistry of Parkinson's disease. *Annual review of biochemistry* 2005;74:29–52.
- Costoya JA. Functional analysis of the role of POK transcriptional repressors. *Briefings in functional genomics & proteomics* 2007;6:8–18. [PubMed: 17384421]
- Dauer W, Przedborski S. Parkinson's disease: mechanisms and models. *Neuron* 2003;39:889–909. [PubMed: 12971891]
- de Kloet ER, Sibug RM, Helmerhorst FM, Schmidt MV. Stress, genes and the mechanism of programming the brain for later life. *Neuroscience and biobehavioral reviews* 2005;29:271–281. [PubMed: 15811498]
- Doucet JP, Nakabeppu Y, Bedard PJ, Hope BT, Nestler EJ, Jasmin BJ, Chen JS, Iadarola MJ, St-Jean M, Wigle N, Blanchet P, Grondin R, Robertson GS. Chronic alterations in dopaminergic neurotransmission produce a persistent elevation of deltaFosB-like protein(s) in both the rodent and primate striatum. *The European journal of neuroscience* 1996;8:365–381. [PubMed: 8714707]
- Duchemin AM, Gudehithlu KP, Neff NH, Hadjiconstantinou M. c-fos mRNA in mouse brain after MPTP treatment. *Neurochemistry international* 1992;20:281–287. [PubMed: 1284678]
- Eberling JL, Bankiewicz KS, Jordan S, VanBrocklin HF, Jagust WJ. PET studies of functional compensation in a primate model of Parkinson's disease. *Neuroreport* 1997;8:2727–2733. [PubMed: 9295108]
- Edwards MG, Sarkar D, Klopp R, Morrow JD, Weindruch R, Prolla TA. Impairment of the transcriptional responses to oxidative stress in the heart of aged C57BL/6 mice. *Annals of the New York Academy of Sciences* 2004;1019:85–95. [PubMed: 15246999]
- Farrer MJ. Genetics of Parkinson disease: paradigm shifts and future prospects. *Nature reviews* 2006;7:306–318.
- Fernandez-Gonzalez A, Perez-Otano I, Morgan JI. MPTP selectively induces haem oxygenase-1 expression in striatal astrocytes. *The European journal of neuroscience* 2000;12:1573–1583. [PubMed: 10792435]
- Francis JW, Von Visger J, Markelonis GJ, Oh TH. Neuroglial responses to the dopaminergic neurotoxicant 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine in mouse striatum. *Neurotoxicology and teratology* 1995;17:7–12. [PubMed: 7535890]
- Gainetdinov RR, Fumagalli F, Jones SR, Caron MG. Dopamine transporter is required for in vivo MPTP neurotoxicity: evidence from mice lacking the transporter. *J Neurochem* 1997;69:1322–1325. [PubMed: 9282960]
- Gallant S, Gilkeson G. ETS transcription factors and regulation of immunity. *Archivum immunologiae et therapiae experimentalis* 2006;54:149–163. [PubMed: 16652219]
- German DC, Nelson EL, Liang CL, Speciale SG, Sinton CM, Sonsalla PK. The neurotoxin MPTP causes degeneration of specific nucleus A8, A9 and A10 dopaminergic neurons in the mouse. *Neurodegeneration* 1996;5:299–312. [PubMed: 9117541]
- Grigoryev DN, Liu M, Cheadle C, Barnes KC, Rabb H. Genomic profiling of kidney ischemia-reperfusion reveals expression of specific alloimmunity-associated genes: Linking "immune" and "nonimmune" injury events. *Transplantation proceedings* 2006;38:3333–3336. [PubMed: 17175265]
- Grottick AJ, Bagnol D, Phillips S, McDonald J, Behan DP, Chalmers DT, Hakak Y. Neurotransmission- and cellular stress-related gene expression associated with prepulse inhibition in mice. *Brain Res Brain Res Rev* 2005;139:153–162.
- Grunblatt E, Mandel S, Maor G, Youdim MB. Gene expression analysis in N-methyl-4-phenyl-1,2,3,6-tetrahydropyridine mice model of Parkinson's disease using cDNA microarray: effect of R-apomorphine. *Journal of neurochemistry* 2001;78:1–12. [PubMed: 11432968]
- Gureviciene I, Gurevicius K, Tanila H. Role of alpha-synuclein in synaptic glutamate release. *Neurobiology of disease* 2007;28:83–89. [PubMed: 17689254]
- Hald A, Lotharius J. Oxidative stress and inflammation in Parkinson's disease: is there a causal link? *Experimental neurology* 2005;193:279–290. [PubMed: 15869932]
- Hald A, Van Beek J, Lotharius J. Inflammation in Parkinson's disease: causative or epiphenomenal? *Sub-cellular biochemistry* 2007;42:249–279. [PubMed: 17612055]

- Hamre K, Tharp R, Poon K, Xiong X, Smeyne RJ. Differential strain susceptibility following 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) administration acts in an autosomal dominant fashion: quantitative analysis in seven strains of *Mus musculus*. *Brain Res Brain Res Rev* 1999;828:91–103.
- Haque ME, Thomas KJ, D'Souza C, Callaghan S, Kitada T, Slack RS, Fraser P, Cookson MR, Tandon A, Park DS. Cytoplasmic Pink1 activity protects neurons from dopaminergic neurotoxin MPTP. *Proc Natl Acad Sci U S A*. 2008
- Hardy RJ, Friedrich VL Jr. Progressive remodeling of the oligodendrocyte process arbor during myelogenesis. *Developmental neuroscience* 1996;18:243–254. [PubMed: 8911764]
- Hasegawa E, Asagami H, Kang D, Minakami S, Takeshige K. 1-Methyl-4-phenylpyridinium (MPP+) inhibits mitochondrial oxygen consumption mediated by succinate as well as malate in rat pheochromocytoma PC12 cells. *Biochemistry and molecular biology international* 1995;35:409–413. [PubMed: 7663396]
- Hebert G, Arsaut J, Dantzer R, Demotes-Mainard J. Time-course of the expression of inflammatory cytokines and matrix metalloproteinases in the striatum and mesencephalon of mice injected with 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine, a dopaminergic neurotoxin. *Neuroscience letters* 2003;349:191–195. [PubMed: 12951201]
- Herkenham M, Little MD, Bankiewicz K, Yang SC, Markey SP, Johannessen JN. Selective retention of MPP+ within the monoaminergic systems of the primate brain following MPTP administration: an in vivo autoradiographic study. *Neuroscience* 1991;40:133–158. [PubMed: 2052148]
- Hershenson MB. p21Waf1/Cip1 and the prevention of oxidative stress. *American journal of physiology* 2004;286:L502–L505. [PubMed: 14761871]
- Huang Y, Cheung L, Rowe D, Halliday G. Genetic contributions to Parkinson's disease. *Brain research* 2004;46:44–70.
- Hunot S, Vila M, Teismann P, Davis RJ, Hirsch EC, Przedborski S, Rakic P, Flavell RA. JNK-mediated induction of cyclooxygenase 2 is required for neurodegeneration in a mouse model of Parkinson's disease. *Proceedings of the National Academy of Sciences of the United States of America* 2004;101:665–670. [PubMed: 14704277]
- Hunter RL, Dragicevic N, Seifert K, Choi DY, Liu M, Kim HC, Cass WA, Sullivan PG, Bing G. Inflammation induces mitochondrial dysfunction and dopaminergic neurodegeneration in the nigrostriatal system. *Journal of neurochemistry* 2007;100:1375–1386. [PubMed: 17254027]
- Imai Y, Inoue H, Kataoka A, Hua-Qin W, Masuda M, Ikeda T, Tsukita K, Soda M, Kodama T, Fuwa T, Honda Y, Kaneko S, Matsumoto S, Wakamatsu K, Ito S, Miura M, Aosaki T, Itoharu S, Takahashi R. Pael receptor is involved in dopamine metabolism in the nigrostriatal system. *Neuroscience research* 2007;59:413–425. [PubMed: 17889953]
- Imai Y, Soda M, Murakami T, Shoji M, Abe K, Takahashi R. A product of the human gene adjacent to parkin is a component of Lewy bodies and suppresses Pael receptor-induced cell death. *The Journal of biological chemistry* 2003;278:51901–51910. [PubMed: 14532270]
- Ishizawa T, Mattila P, Davies P, Wang D, Dickson DW. Colocalization of tau and alpha-synuclein epitopes in Lewy bodies. *Journal of neuropathology and experimental neurology* 2003;62:389–397. [PubMed: 12722831]
- Ito D, Imai Y, Ohsawa K, Nakajima K, Fukuuchi Y, Kohsaka S. Microglia-specific localization of a novel calcium binding protein, Iba1. *Brain Res Brain Res Rev* 1998;57:1–9.
- Iwamoto R, Higashiyama S, Mitamura T, Taniguchi N, Klagsbrun M, Mekada E. Heparin-binding EGF-like growth factor, which acts as the diphtheria toxin receptor, forms a complex with membrane protein DRAP27/CD9, which up-regulates functional receptors and diphtheria toxin sensitivity. *The EMBO journal* 1994;13:2322–2330. [PubMed: 8194524]
- Jackson-Lewis V, Jakowec M, Burke RE, Przedborski S. Time course and morphology of dopaminergic neuronal death caused by the neurotoxin 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine. *Neurodegeneration* 1995;4:257–269. [PubMed: 8581558]
- Jackson-Lewis V, Smeyne RJ. MPTP and SNpc DA neuronal vulnerability: role of dopamine, superoxide and nitric oxide in neurotoxicity. *Minireview. Neurotoxicity research* 2005;7:193–202.
- Jakes R, Spillantini MG, Goedert M. Identification of two distinct synucleins from human brain. *FEBS letters* 1994;345:27–32. [PubMed: 8194594]

- Jakovcevski I, Zecevic N. Sequence of oligodendrocyte development in the human fetal telencephalon. *Glia* 2005;49:480–491. [PubMed: 15578660]
- Javitch JA, D'Amato RJ, Strittmatter SM, Snyder SH. Parkinsonism-inducing neurotoxin, N-methyl-4-phenyl-1,2,3,6-tetrahydropyridine: uptake of the metabolite N-methyl-4-phenylpyridine by dopamine neurons explains selective toxicity. *Proc Natl Acad Sci U S A* 1985;82:2173–2177. [PubMed: 3872460]
- Kawaguchi Y, Wilson CJ, Augood SJ, Emson PC. Striatal interneurons: chemical, physiological and morphological characterization. *Trends in neurosciences* 1995;18:527–535. [PubMed: 8638293]
- Kindt MV, Heikkila RE, Nicklas WJ. Mitochondrial and metabolic toxicity of 1-methyl-4-(2'-methylphenyl)-1,2,3,6-tetrahydropyridine. *The Journal of pharmacology and experimental therapeutics* 1987;242:858–863. [PubMed: 3498818]
- Kingsbury AE, Daniel SE, Sangha H, Eisen S, Lees AJ, Foster OJ. Alteration in alpha-synuclein mRNA expression in Parkinson's disease. *Mov Disord* 2004;19:162–170. [PubMed: 14978671]
- Kitada T, Asakawa S, Hattori N, Matsumine H, Yamamura Y, Minoshima S, Yokochi M, Mizuno Y, Shimizu N. Mutations in the parkin gene cause autosomal recessive juvenile parkinsonism. *Nature* 1998;392:605–608. [PubMed: 9560156]
- Kitada T, Pisani A, Porter DR, Yamaguchi H, Tscherter A, Martella G, Bonsi P, Zhang C, Pothos EN, Shen J. Impaired dopamine release and synaptic plasticity in the striatum of PINK1-deficient mice. *Proc Natl Acad Sci U S A* 2007;104:11441–11446. [PubMed: 17563363]
- Kitao Y, Imai Y, Ozawa K, Kataoka A, Ikeda T, Soda M, Nakimawa K, Kiyama H, Stern DM, Hori O, Wakamatsu K, Ito S, Itohara S, Takahashi R, Ogawa S. Pael receptor induces death of dopaminergic neurons in the substantia nigra via endoplasmic reticulum stress and dopamine toxicity, which is enhanced under condition of parkin inactivation. *Human molecular genetics* 2007;16:50–60. [PubMed: 17116640]
- Kohutnicka M, Lewandowska E, Kurkowska-Jastrzebska I, Czlonkowski A, Czlonkowska A. Microglial and astrocytic involvement in a murine model of Parkinson's disease induced by 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP). *Immunopharmacology* 1998;39:167–180. [PubMed: 9754903]
- Krueger MJ, Singer TP, Casida JE, Ramsay RR. Evidence that the blockade of mitochondrial respiration by the neurotoxin 1-methyl-4-phenylpyridinium (MPP+) involves binding at the same site as the respiratory inhibitor, rotenone. *Biochemical and biophysical research communications* 1990;169:123–128. [PubMed: 2350337]
- Kruger R, Kuhn W, Muller T, Woitalla D, Graeber M, Kosel S, Przuntek H, Epplen JT, Schols L, Riess O. Ala30Pro mutation in the gene encoding alpha-synuclein in Parkinson's disease. *Nature genetics* 1998;18:106–108. [PubMed: 9462735]
- Kuhn K, Wellen J, Link N, Maskri L, Lubbert H, Stichel CC. The mouse MPTP model: gene expression changes in dopaminergic neurons. *The European journal of neuroscience* 2003;17:1–12. [PubMed: 12534964]
- Ladeby R, Wirenfeldt M, Dalmau I, Gregersen R, Garcia-Ovejero D, Babcock A, Owens T, Finsen B. Proliferating resident microglia express the stem cell antigen CD34 in response to acute neural injury. *Glia* 2005;50:121–131. [PubMed: 15657941]
- Landrigan PJ, Sonawane B, Butler RN, Trasande L, Callan R, Droller D. Early environmental origins of neurodegenerative disease in later life. *Environmental health perspectives* 2005;113:1230–1233. [PubMed: 16140633]
- Leung YF, Tam PO, Lee WS, Lam DS, Yam HF, Fan BJ, Tham CC, Chua JK, Pang CP. The dual role of dexamethasone on anti-inflammation and outflow resistance demonstrated in cultured human trabecular meshwork cells. *Molecular vision* 2003;9:425–439. [PubMed: 12963864]
- Li JY, Henning Jensen P, Dahlstrom A. Differential localization of alpha-, beta- and gamma-synucleins in the rat CNS. *Neuroscience* 2002;113:463–478. [PubMed: 12127102]
- Li XJ, Li SH, Sharp AH, Nucifora FC Jr, Schilling G, Lanahan A, Worley P, Snyder SH, Ross CA. A huntingtin-associated protein enriched in brain with implications for pathology. *Nature* 1995;378:398–402. [PubMed: 7477378]
- Linder JC, Young SJ, Groves PM. Electron microscopic evidence for neurotoxicity in the basal ganglia. *Neurochem Int* 1995;26:195–202. [PubMed: 7599540]

- Liu PK, Grossman RG, Hsu CY, Robertson CS. Ischemic injury and faulty gene transcripts in the brain. *Trends in neurosciences* 2001;24:581–588. [PubMed: 11576672]
- Malagelada C, Ryu EJ, Biswas SC, Jackson-Lewis V, Greene LA. RTP801 is elevated in Parkinson brain substantia nigral neurons and mediates death in cellular models of Parkinson's disease by a mechanism involving mammalian target of rapamycin inactivation. *J Neurosci* 2006;26:9996–10005. [PubMed: 17005863]
- Mandel S, Grunblatt E, Maor G, Youdim MB. Early and late gene changes in MPTP mice model of Parkinson's disease employing cDNA microarray. *Neurochemical research* 2002;27:1231–1243. [PubMed: 12462421]
- Marazziti D, Gallo A, Golini E, Matteoni R, Tocchini-Valentini GP. Molecular cloning and chromosomal localization of the mouse Gpr37 gene encoding an orphan G-protein-coupled peptide receptor expressed in brain and testis. *Genomics* 1998;53:315–324. [PubMed: 9799598]
- Marazziti D, Golini E, Gallo A, Lombardi MS, Matteoni R, Tocchini-Valentini GP. Cloning of GPR37, a gene located on chromosome 7 encoding a putative G-protein-coupled peptide receptor, from a human frontal brain EST library. *Genomics* 1997;45:68–77. [PubMed: 9339362]
- Marin O, Anderson SA, Rubenstein JL. Origin and molecular specification of striatal interneurons. *J Neurosci* 2000;20:6063–6076. [PubMed: 10934256]
- McClintick JN, Edenberg HJ. Effects of filtering by Present call on analysis of microarray experiments. *BMC bioinformatics* 2006;7:49. [PubMed: 16448562]
- McGeer PL, Itagaki S, Boyes BE, McGeer EG. Reactive microglia are positive for HLA-DR in the substantia nigra of Parkinson's and Alzheimer's disease brains. *Neurology* 1988;38:1285–1291. [PubMed: 3399080]
- McGeer PL, McGeer EG. Glial reactions in Parkinson's disease. *Mov Disord.* 2007
- Mead RJ, Neal JW, Griffiths MR, Linington C, Botto M, Lassmann H, Morgan BP. Deficiency of the complement regulator CD59a enhances disease severity, demyelination and axonal injury in murine acute experimental allergic encephalomyelitis. *Laboratory investigation; a journal of technical methods and pathology* 2004;84:21–28. [PubMed: 14631387]
- Miller RM, Chen LL, Kiser GL, Giesler TL, Kaysser-Kranich TM, Palaniappan C, Federoff HJ. Temporal evolution of mouse striatal gene expression following MPTP injury. *Neurobiology of aging* 2005;26:765–775. [PubMed: 15708451]
- Mootha VK, Lindgren CM, Eriksson KF, Subramanian A, Sihag S, Lehar J, Puigserver P, Carlsson E, Ridderstrale M, Laurila E, Houstis N, Daly MJ, Patterson N, Mesirov JP, Golub TR, Tamayo P, Spiegelman B, Lander ES, Hirschhorn JN, Altshuler D, Groop LC. PGC-1 α -responsive genes involved in oxidative phosphorylation are coordinately downregulated in human diabetes. *Nature genetics* 2003;34:267–273. [PubMed: 12808457]
- Morgan JI, Cohen DR, Hempstead JL, Curran T. Mapping patterns of c-fos expression in the central nervous system after seizure. *Science* 1987;237:192–197. [PubMed: 3037702]
- Morgan JI, Curran T. Stimulus-transcription coupling in neurons: role of cellular immediate-early genes. *Trends in neurosciences* 1989;12:459–462. [PubMed: 2479148]
- Murakami T, Shoji M, Imai Y, Inoue H, Kawarabayashi T, Matsubara E, Harigaya Y, Sasaki A, Takahashi R, Abe K. Pael-R is accumulated in Lewy bodies of Parkinson's disease. *Annals of neurology* 2004;55:439–442. [PubMed: 14991825]
- Nagatsu T, Mogi M, Ichinose H, Togari A. Cytokines in Parkinson's disease. *J Neural Transm Suppl* 2000;143–151. [PubMed: 11128604]
- Nagatsu T, Sawada M. Inflammatory process in Parkinson's disease: role for cytokines. *Current pharmaceutical design* 2005;11:999–1016. [PubMed: 15777250]
- Nurmi E, Ruottinen HM, Bergman J, Haaparanta M, Solin O, Sonninen P, Rinne JO. Rate of progression in Parkinson's disease: a 6-[18F]fluoro-L-dopa PET study. *Mov Disord* 2001;16:608–615. [PubMed: 11481683]
- O'Callaghan JP, Miller DB. Neurotoxicity profiles of substituted amphetamines in the C57BL/6J mouse. *The Journal of pharmacology and experimental therapeutics* 1994;270:741–751. [PubMed: 8071867]
- O'Reilly MA. Redox activation of p21Cip1/WAF1/Sdi1: a multifunctional regulator of cell survival and death. *Antioxidants & redox signaling* 2005;7:108–118. [PubMed: 15650400]

- Ou YH, Chung PH, Hsu FF, Sun TP, Chang WY, Shieh SY. The candidate tumor suppressor BTG3 is a transcriptional target of p53 that inhibits E2F1. *The EMBO journal* 2007;26:3968–3980. [PubMed: 17690688]
- Oyake T, Itoh K, Motohashi H, Hayashi N, Hoshino H, Nishizawa M, Yamamoto M, Igarashi K. Bach proteins belong to a novel family of BTB-basic leucine zipper transcription factors that interact with MafK and regulate transcription through the NF-E2 site. *Molecular and cellular biology* 1996;16:6083–6095. [PubMed: 8887638]
- Pattarini R, Smeyne RJ, Morgan JI. Temporal mRNA profiles of inflammatory mediators in the murine 1-methyl-4-phenyl-1,2,3,6-tetrahydropyrimidine model of Parkinson's disease. *Neuroscience* 2007;145:654–668. [PubMed: 17258864]
- Perez-Otano I, Mandelzys A, Morgan JI. MPTP-Parkinsonism is accompanied by persistent expression of a delta-FosB-like protein in dopaminergic pathways. *Brain research* 1998;53:41–52. [PubMed: 9473580]
- Petit A, Kawarai T, Paitel E, Sanjo N, Maj M, Scheid M, Chen F, Gu Y, Hasegawa H, Salehi-Rad S, Wang L, Rogaeva E, Fraser P, Robinson B, St George-Hyslop P, Tandon A. Wild-type PINK1 prevents basal and induced neuronal apoptosis, a protective effect abrogated by Parkinson disease-related mutations. *The Journal of biological chemistry* 2005;280:34025–34032. [PubMed: 16079129]
- Polymeropoulos MH, Lavedan C, Leroy E, Ide SE, Dehejia A, Dutra A, Pike B, Root H, Rubenstein J, Boyer R, Stenroos ES, Chandrasekharappa S, Athanassiadou A, Papapetropoulos T, Johnson WG, Lazzarini AM, Duvoisin RC, Di Iorio G, Golbe LI, Nussbaum RL. Mutation in the alpha-synuclein gene identified in families with Parkinson's disease. *Science* 1997;276:2045–2047. [PubMed: 9197268]
- Pridgeon JW, Olzmann JA, Chin LS, Li L. PINK1 Protects against Oxidative Stress by Phosphorylating Mitochondrial Chaperone TRAP1. *PLoS biology* 2007;5:e172. [PubMed: 17579517]
- Przedborski S, Vila M. The 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine mouse model: a tool to explore the pathogenesis of Parkinson's disease. *Annals of the New York Academy of Sciences* 2003;991:189–198. [PubMed: 12846987]
- Qian YM, Qin X, Miwa T, Sun X, Halperin JA, Song WC. Identification and functional characterization of a new gene encoding the mouse terminal complement inhibitor CD59. *J Immunol* 2000;165:2528–2534. [PubMed: 10946279]
- Quackenbush J. Computational analysis of microarray data. *Nature reviews* 2001;2:418–427.
- Reichard JF, Motz GT, Puga A. Heme oxygenase-1 induction by NRF2 requires inactivation of the transcriptional repressor BACH1. *Nucleic Acids Res.* 2007
- Rescher U, Gerke V. S100A10/p11: family, friends and functions. *Pflugers Arch* 2008;455:575–582. [PubMed: 17638009]
- Rety S, Sopkova J, Renouard M, Osterloh D, Gerke V, Tabaries S, Russo-Marie F, Lewit-Bentley A. The crystal structure of a complex of p11 with the annexin II N-terminal peptide. *Nature structural biology* 1999;6:89–95.
- Rinne OJ, Nurmi E, Ruottinen HM, Bergman J, Eskola O, Solin O. [(18)F]FDOPA and [(18)F]CFT are both sensitive PET markers to detect presynaptic dopaminergic hypofunction in early Parkinson's disease. *Synapse* 2001;40:193–200. [PubMed: 11304757]
- Rocke DM, Durbin B. Approximate variance-stabilizing transformations for gene-expression microarray data. *Bioinformatics (Oxford, England)* 2003;19:966–972.
- Rollema H, Kuhr WG, Kranenborg G, De Vries J, Van den Berg C. MPP+-induced efflux of dopamine and lactate from rat striatum have similar time courses as shown by in vivo brain dialysis. *The Journal of pharmacology and experimental therapeutics* 1988;245:858–866. [PubMed: 2455037]
- Santamaria-Kisiel L, Rintala-Dempsey AC, Shaw GS. Calcium-dependent and -independent interactions of the S100 protein family. *The Biochemical journal* 2006;396:201–214. [PubMed: 16683912]
- Schapira AH. Etiology of Parkinson's disease. *Neurology* 2006;66:S10–S23. [PubMed: 16717248]
- Schapira AH. Mitochondria in the aetiology and pathogenesis of Parkinson's disease. *Lancet neurology* 2008;7:97–109. [PubMed: 18093566]
- Schmidt J, Mertz K, Morgan JI. Regulation of heme oxygenase-1 expression by dopamine in cultured C6 glioma and primary astrocytes. *Brain Res Brain Res Rev* 1999;73:50–59.

- Schoenebeck B, Bader V, Zhu XR, Schmitz B, Lubbert H, Stichel CC. Sgk1, a cell survival response in neurodegenerative diseases. *Molecular and cellular neurosciences* 2005;30:249–264. [PubMed: 16125969]
- Schreiber SS, Tocco G, Shors TJ, Thompson RF. Activation of immediate early genes after acute stress. *Neuroreport* 1991;2:17–20. [PubMed: 1768844]
- Scolding NJ, Frith S, Linington C, Morgan BP, Campbell AK, Compston DA. Myelin-oligodendrocyte glycoprotein (MOG) is a surface marker of oligodendrocyte maturation. *Journal of neuroimmunology* 1989;22:169–176. [PubMed: 2649509]
- Serdobova I, Pla M, Reichenbach P, Sperisen P, Ghysdael J, Wilson A, Freeman J, Nabholz M. Elf-1 contributes to the function of the complex interleukin (IL)-2-responsive enhancer in the mouse IL-2 receptor alpha gene. *The Journal of experimental medicine* 1997;185:1211–1221. [PubMed: 9104808]
- Shen YQ, Hebert G, Lin LY, Luo YL, Moze E, Li KS, Neveu PJ. Interleukine-1beta and interleukine-6 levels in striatum and other brain structures after MPTP treatment: influence of behavioral lateralization. *Journal of neuroimmunology* 2005;158:14–25. [PubMed: 15589033]
- Shim KS, Rosner M, Freilinger A, Lubec G, Hengstschlager M. Bach2 is involved in neuronal differentiation of N1E-115 neuroblastoma cells. *Experimental cell research* 2006;312:2264–2278. [PubMed: 16631738]
- Smeyne RJ, Jackson-Lewis V. The MPTP model of Parkinson's disease. *Brain research* 2005;134:57–66. [PubMed: 15790530]
- Smith TS, Trimmer PA, Khan SM, Tinklepaugh DL, Bennett JP Jr. Mitochondrial toxins in models of neurodegenerative diseases. II: Elevated zif268 transcription and independent temporal regulation of striatal D1 and D2 receptor mRNAs and D1 and D2 receptor-binding sites in C57BL/6 mice during MPTP treatment. *Brain research* 1997;765:189–197. [PubMed: 9313891]
- Sriram K, Benkovic SA, Hebert MA, Miller DB, O'Callaghan JP. Induction of gp130-related cytokines and activation of JAK2/STAT3 pathway in astrocytes precedes up-regulation of glial fibrillary acidic protein in the 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine model of neurodegeneration: key signaling pathway for astrogliosis in vivo? *The Journal of biological chemistry* 2004;279:19936–19947. [PubMed: 14996842]
- Sriram K, Matheson JM, Benkovic SA, Miller DB, Luster MI, O'Callaghan JP. Deficiency of TNF receptors suppresses microglial activation and alters the susceptibility of brain regions to MPTP-induced neurotoxicity: role of TNF-alpha. *Faseb J* 2006;20:670–682. [PubMed: 16581975]
- Stichel CC, Augustin M, Kuhn K, Zhu XR, Engels P, Ullmer C, Lubbert H. Parkin expression in the adult mouse brain. *The European journal of neuroscience* 2000;12:4181–4194. [PubMed: 11122330]
- Stichel CC, Schoenebeck B, Foguet M, Siebertz B, Bader V, Zhu XR, Lubbert H. sgk1, a member of an RNA cluster associated with cell death in a model of Parkinson's disease. *The European journal of neuroscience* 2005;21:301–316. [PubMed: 15673431]
- Su X, Maguire-Zeiss KA, Giuliano R, Prifti L, Venkatesh K, Federoff HJ. Synuclein activates microglia in a model of Parkinson's disease. *Neurobiology of aging*. 2007
- Subramanian A, Kuehn H, Gould J, Tamayo P, Mesirov JP. GSEA-P: a desktop application for Gene Set Enrichment Analysis. *Bioinformatics (Oxford, England)* 2007;23:3251–3253.
- Subramanian A, Tamayo P, Mootha VK, Mukherjee S, Ebert BL, Gillette MA, Paulovich A, Pomeroy SL, Golub TR, Lander ES, Mesirov JP. Gene set enrichment analysis: a knowledge-based approach for interpreting genome-wide expression profiles. *Proceedings of the National Academy of Sciences of the United States of America* 2005;102:15545–15550. [PubMed: 16199517]
- Sundstrom E, Stromberg I, Tsutsumi T, Olson L, Jonsson G. Studies on the effect of 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) on central catecholamine neurons in C57BL/6 mice. Comparison with three other strains of mice. *Brain research* 1987;405:26–38. [PubMed: 2882814]
- Tan EK, Yew K, Chua E, Puvan K, Shen H, Lee E, Puong KY, Zhao Y, Pavanni R, Wong MC, Jamora D, de Silva D, Moe KT, Woon FP, Yuen Y, Tan L. PINK1 mutations in sporadic early-onset Parkinson's disease. *Mov Disord* 2006;21:789–793. [PubMed: 16482571]
- Taymans JM, Van den Haute C, Baekelandt V. Distribution of PINK1 and LRRK2 in rat and mouse brain. *Journal of neurochemistry* 2006;98:951–961. [PubMed: 16771836]

- Tokumitsu H, Mizutani A, Minami H, Kobayashi R, Hidaka H. A calyculin-associated protein is a newly identified member of the Ca²⁺/phospholipid-binding proteins, annexin family. *The Journal of biological chemistry* 1992;267:8919–8924. [PubMed: 1533622]
- Unoki M, Nakamura Y. Growth-suppressive effects of BPOZ and EGR2, two genes involved in the PTEN signaling pathway. *Oncogene* 2001;20:4457–4465. [PubMed: 11494141]
- Valente EM, Abou-Sleiman PM, Caputo V, Muqit MM, Harvey K, Gispert S, Ali Z, Del Turco D, Bentivoglio AR, Healy DG, Albanese A, Nussbaum R, Gonzalez-Maldonado R, Deller T, Salvi S, Cortelli P, Gilks WP, Latchman DS, Harvey RJ, Dallapiccola B, Auburger G, Wood NW. Hereditary early-onset Parkinson's disease caused by mutations in PINK1. *Science (New York, NY)* 2004;304:1158–1160.
- Vila M, Jackson-Lewis V, Vukosavic S, Djaldetti R, Liberatore G, Offen D, Korsmeyer SJ, Przedborski S. Bax ablation prevents dopaminergic neurodegeneration in the 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine mouse model of Parkinson's disease. *Proceedings of the National Academy of Sciences of the United States of America* 2001;98:2837–2842. [PubMed: 11226327]
- Wakabayashi K, Tanji K, Mori F, Takahashi H. The Lewy body in Parkinson's disease: molecules implicated in the formation and degradation of alpha-synuclein aggregates. *Neuropathology* 2007;27:494–506. [PubMed: 18018486]
- Wilms H, Zecca L, Rosenstiel P, Sievers J, Deuschl G, Lucius R. Inflammation in Parkinson's diseases and other neurodegenerative diseases: cause and therapeutic implications. *Current pharmaceutical design* 2007;13:1925–1928. [PubMed: 17584117]
- Wood-Kaczmar A, Gandhi S, Wood NW. Understanding the molecular causes of Parkinson's disease. *Trends Mol Med* 2006;12:521–528. [PubMed: 17027339]
- Xie X, Lu J, Kulbokas EJ, Golub TR, Mootha V, Lindblad-Toh K, Lander ES, Kellis M. Systematic discovery of regulatory motifs in human promoters and 3' UTRs by comparison of several mammals. *Nature* 2005;434:338–345. [PubMed: 15735639]
- Yuan Y, Jin J, Yang B, Zhang W, Hu J, Zhang Y, Chen NH. Overexpressed Alpha-Synuclein Regulated the Nuclear Factor-kappaB Signal Pathway. *Cellular and molecular neurobiology*. 2007
- Zeng Z, Su K, Kyaw H, Li Y. A novel endothelin receptor type-B-like gene enriched in the brain. *Biochemical and biophysical research communications* 1997;233:559–567. [PubMed: 9144577]
- Zhang XL, Zhang D, Michel FJ, Blum JL, Simmen FA, Simmen RC. Selective interactions of Kruppel-like factor 9/basic transcription element-binding protein with progesterone receptor isoforms A and B determine transcriptional activity of progesterone-responsive genes in endometrial epithelial cells. *The Journal of biological chemistry* 2003;278:21474–21482. [PubMed: 12672823]
- Zhou H, Falkenburger BH, Schulz JB, Tieu K, Xu Z, Xia XG. Silencing of the Pink1 gene expression by conditional RNAi does not induce dopaminergic neuron death in mice. *International journal of biological sciences* 2007;3:242–250. [PubMed: 17389931]

Figure 1. Temporal profile of mRNA responses to MPTP in the striatum of C57BL/6J mice

Animals were injected with either MPTP (20 mg/kg) or saline (control) every two hours for a total of 4 injections. Striata were harvested at 5 (3 injections), 24 and 72 hours. Total RNA was hybridized to the Affymetrix Mouse Genome 430 2.0 array and results analyzed as described in Materials and Methods. Hierarchical cluster analysis reveals three discrete cohorts of probesets modulated at each time point. Only a small fraction of probesets are regulated at multiple time points. Each vertical column represents a single mouse treated as indicated in the figure and each horizontal row is an individual probeset. Probesets that are upregulated in treated compared to control mice appear in red, those that are downregulated appear in green. The relative log₂(ratio) is reflected by the intensity of the color. A total of 47 animals, 23 treated with saline and 24 treated with MPTP, were used. The data reveals three general phases of gene expression changes (early, intermediate and late), that are constituted by relatively unique clusters of probesets.

Figure 2. Quantitative assessment of mRNA changes in the striatum of MPTP-treated C57BL/6J mice

MPTP sensitive (C57BL/6J) mice were injected at time zero with a single dose of MPTP (40 mg/kg) and sacrificed at 2, 5, 12 and 24 hr and 3, 7, 8, 9, 10 and 11 days. Levels of mRNA for candidate genes of the early (*Cdkn1a*, *Nfkb1a*, *Fosb*, *Nr4a1* and *Gadd45b*) and intermediate (*Hmox1*, *Pdlim4*, *Edg3*, *Tnfrsf12a* and *Hbegf*) response phases were evaluated by quantitative RT-PCR. Results are expressed as the number of copies of a specific mRNA (normalized versus the number of copies of *Gapdh* mRNA) versus time (hours). Data are presented as mean $\pm$ S.E.M. of 6 (control) and 3 (MPTP-treated) animals. Differences versus control (time point zero) were analyzed with one way ANOVA and Bonferroni post-hoc test and are indicated

with asterisks at the top of each significantly different point (** $P < 0.001$, * $P < 0.01$, * $P < 0.05$). Results are in accordance with those obtained by Affymetrix gene chip technology.

Figure 3. Hierarchical cluster analysis reveals differences between the early (5 hr) and intermediate (24 hr) MPTP-induced responses in striatum, cortex and cerebellum of C57BL/6J mice

Animals were injected with either MPTP (20 mg/kg) or saline (control) every two hours for a total of 4 injections and striatum, cortex and cerebellum harvested at 5 (3 injections) and 24 hours. Total RNA was hybridized to the Affymetrix Mouse Genome 430 2.0 array and results analyzed as described in Materials and Methods. In this hierarchical cluster analysis each vertical column represents a single mouse treated as indicated on top of the figure and each horizontal row is an individual probeset. Probesets that are upregulated in treated compared to control mice appear in red, those that are downregulated appear in green. The relative log₂ (ratio) is reflected by the intensity of the color. A total of 10 animals, 4 treated with saline and 6 treated with MPTP, were used. The visual pattern generated by the hierarchical cluster analysis program depends on the number and type of samples used for the analysis. Therefore, similar time points, may display visually different patterns in different figures although the genes within the clusters are identical. Whereas the early phase occurs to various extents in all three brain regions, the intermediate phase is restricted to the striatum.

Figure 4. The MPTP-induced intermediate phase, but not the early phase differ between MPTP-sensitive (C57BL/6J) and MPTP-resistant (SWR) strains of mice

C57BL/6J and SWR mice were injected with either MPTP (20 mg/kg) or saline (control) every two hours for a total of 4 injections. Striatum was harvested at 5 (3 injections) and 24 hours, and total RNA was isolated and hybridized onto the Affymetrix Mouse Genome 430 2.0 array. The expression profile of the probesets previously identified in C57BL/6J mice (Figure 1 and Supplemental table 1) was compared to a new cohort of MPTP-sensitive (C57BL/6J) mice as well as to a cohort of MPTP-resistant (SWR) mice at 5 and 24 hours after treatment to generate this heat map. Each vertical column represents a single mouse treated as indicated on top of the figure and each horizontal row is an individual probeset. Probesets that are upregulated in treated compared to control mice appear in red, those that are downregulated appear in green. The relative log₂ (ratio) is reflected by the intensity of the color. A total of 19 C57BL/6J and 24 SWR mice (9 C57BL/6J and 11 SWR treated with saline; 10 C57BL/6J and 13 SWR treated with MPTP) were used to perform the experiments described in this figure. The visual pattern generated by the hierarchical cluster analysis program depends on the number and type of samples used for the analysis. Therefore, similar time points, may display visually different patterns in different figures although the genes within the clusters are identical. Whereas the early phase is indistinguishable in MPTP-sensitive (C57BL/6J) and MPTP-resistant (SWR) strains of mice, the intermediate phase is attenuated in resistant SWR mice.

Figure 5. Quantitative assessment of differences between early and intermediate mRNA responses in MPTP-sensitive (C57BL/6J) and MPTP-resistant (SWR) mice by qRT-PCR

MPTP-sensitive (C57BL/6J) and MPTP-resistant (SWR) mice were injected at time zero with a single dose of MPTP (40 mg/kg) and sacrificed at 2, 5 and 24 hr. Levels of mRNA for candidate genes of the early (*Cdkn1a*, *Nfkb1a*, *Fosb*, *Nr4a1* and *Gadd45b*) and intermediate (*Hmox1*, *Pdlim4*, *Edg3*, *Tnfrsf12a* and *Hbegf*) response phases were evaluated by quantitative RT-PCR. Results are expressed as the number of copies of a specific mRNA (normalized versus the number of copies of *Gapdh* mRNA) versus time (hours). Data are presented as mean $\pm$ S.E.M. of 3 (Ctrl, 2 and 5 hr time points) or 4 (24 hr time point) animals. Differences between strains were analyzed by two way ANOVA followed by Bonferroni post-hoc test and

significance levels are indicated with letters at the bottom of each pair of columns (A indicates $P < 0.001$, B indicates $P < 0.01$). Differences versus control (time point zero) within a single strain were analyzed with one way ANOVA and Bonferroni post-hoc test and statistical significances indicated with asterisks on top of each column (*** $P < 0.001$, ** $P < 0.01$, * $P < 0.05$). Results are in agreement with those obtained by Affymetrix gene chip technology and indicate no quantitative differences in the early response, whereas the intermediate response is attenuated in SWR mice compared to C57BL/6J mice.

Figure 6. Hierarchical cluster analysis of basal gene expression differences in striata of MPTP-sensitive (C57BL/6J) and MPTP-resistant (SWR) mice

Striatum from untreated animals of both MPTP-sensitive (C57BL/6J) and MPTP-resistant (SWR) mice were harvested and RNA extracted and hybridized onto the Affymetrix Mouse Genome 430 2.0 array to identify genes with differential expression between the two strains. Each vertical column represents a single mouse of the strain indicated on top of the figure (a total of 18 animals, 9 for each strain, were used) and each horizontal row is an individual probeset. Probesets for one strain that are elevated with respect to the other strain appear in red whereas those that are reduced appear in green. The relative $\log_2$ (ratio) is reflected by the intensity of the color. 364 probesets are differentially expressed between sensitive (C57BL/6J) and resistant (SWR) strains of mice.

Figure 7. Quantitative assessment of basal gene expression in striata of MPTP-sensitive (C57BL/6J) and MPTP-resistant (SWR) mice by qRT-PCR

Levels of mRNA for 5 candidate genes (*Kcjn10*, *Comt*, *Apod*, *Pink1* and *Agxt211*) previously identified as being differentially expressed between MPTP-sensitive (C57BL/6J) and MPTP-insensitive (SWR) mice using Affymetrix gene chip technology, were assessed by qRT-PCR. Results are expressed as the number of copies of a specific mRNA normalized versus the number of copies of *Gapdh* mRNA. Data are presented as mean $\pm$ S.E.M. of five animals. Differences between strains were analyzed by Student's t-test and significance levels are indicated with asterisks at the top of each column (*** $P < 0.001$, ** $P < 0.01$, * $P < 0.05$). These data confirm the relative strain-dependent differences revealed by microarray.

Figure 8. The intermediate response in Bax knockout mice does not differ from that in wild type littermates

Bax null mice on a C57BL/6J background and wild type littermates were injected with either MPTP (20 mg/kg) or saline (control) every two hours for a total of 4 injections. Striatum was harvested at 24 hours, and total RNA isolated and hybridized onto the Affymetrix Mouse Genome 430 2.0 array. The expression profile of the probesets previously identified in C57BL/6J mice (Figure 1 and Supplemental table 1) was compared in a cohort of Bax^{-/-} and wild type littermates 24 hours after treatment with MPTP to generate this heat map. Each vertical column represents a single mouse treated as indicated on top of the figure and each horizontal row is an individual probeset. Probesets that are upregulated in treated compared to control mice appear in red, those that are downregulated appear in green. The relative log₂ (ratio) is reflected by the intensity of the color. A total of 20 animals (3 female and 3 male wild type and 2 female and 2 male Bax^{-/-} mice were treated with saline and 5 males of each genotype were treated with MPTP) were used to perform these experiments. The transcriptional response did not differ between the two genotypes, although Bax^{-/-} mice are resistant to MPTP.

Figure 9. Quantitative assessment of mRNA levels in the intermediate response in *Bax* $-/-$ and wild type littermate mice by qRT-PCR

Animals of both strains were injected with either MPTP (20 mg/kg) or saline (control) every two hours for a total of 4 injections. Levels of mRNA for candidate genes of the intermediate transcriptional phase (*Hmox1*, *Pdlim4*, *Edg3*, *Tnfrsf12a* and *Hbegf*) were evaluated by quantitative RT-PCR. Results are expressed as the number of copies of a specific mRNA normalized versus the number of copies of *Gapdh* mRNA. Data are presented as mean $\pm$ S.E.M. of 6 wild type and 4 *Bax* $-/-$ mice treated with saline and 5 MPTP treated mice of both strains. Differences were analyzed with one way ANOVA and Bonferroni post-test and significance values are indicated with asterisks (***) $P < 0.001$, ** $P < 0.01$, * $P < 0.05$). Results are in

accordance with those obtained by Affymetrix gene chip technology and show no significant difference in the expression levels of selected transcripts between wildtype and Bax^{-/-} mice.

Table 1

List of primers and probes for qRT-PCR

Gene Name	mRNA ID #	Forward Primer	Probe	Reverse Primer
<i>Agr21l</i>	NM_027907	5'-CCCTGGCGCTGAGGG-3'	5'-Fam-CACATCGGGCCCTCGTGCAA-BHQ-3'	5'-TGGGATCCGCGGCA-3'
<i>Apod</i>	NM_007470	5'-AACGTCTCAGAGCCAGCCA-3'	5'-Fam-AAGTCCAGTTCTTCCGTTGATGCCA-BHQ-3'	5'-AGGATCCAGTAGGTGCCG-3'
<i>Cdkn1a</i>	NM_007669	5'-TTCCGCACAGAGCAAACT-3'	5'-Fam-CCGTTGTCTTCCGTCCTGG-BHQ-3'	5'-CGCGCAACTGCTCACT-3'
<i>Comt</i>	NM_007744	5'-AGAGAAGGAGTGGGCCATGA-3'	5'-Fam-CGTGGGTGACGAAAAGGCCAA-BHQ-3'	5'-CGGAATCACTGGATCCATGA-3'
<i>Edg3</i>	NM_010101	5'-AACCACGCCAGATGCG-3'	5'-Fam-TTGCAGAACGAGAGCCCTATTTCACACTTTC-BHQ-3'	5'-CGGTGCAGGCCGAC-3'
<i>Nfkbia</i>	NM_010907	5'-CAGCTCACGGAGGACGGA-3'	5'-Fam-ACTCGTTCTGCACTTGGCAATCATCC-BHQ-3'	5'-ATGGTCAGCGGCTTCTCTTC-3'
<i>Fosb</i>	NM_008036	5'-CCAGAGCCAGCCCTAGAAAGAC-3'	5'-Fam-TCGCAGAGCGGAAACAAG-BHQ-3'	5'-CCGACGGTTCCTGCACCTTA-3'
<i>Gadd45b</i>	NM_008655	5'-AGGCGGCCAAACTGATGA-3'	5'-Fam-TGTGACCCGACAGCGTGG-BHQ-3'	5'-CATCTCCTCTTCTCGTCTATGG-3'
<i>Gadph</i>	NM_001001303	5'-TGGATCTGACGTGCCGC-3'	5'-Fam-TGGAGAACTGCCCAGTATGACATCA-BHQ-3'	5'-TGCCTGCTTACACACCTTC-3'
<i>Hbgef</i>	NM_010415	5'-TGCTGCGCTCGGTGATG-3'	5'-Fam-TGAAGCTCTTCTGGCCGAGTGTG-BHQ-3'	5'-ACCGGTCAACCAACGCG-3'
<i>Hmox1</i>	NM_010442	5'-GTGATGGAGCGTCCACAGC-3'	5'-Fam-CGACAGCATGCCCCAGGATTGTC-BHQ-3'	5'-TGGTGGCTCCTTCAAGG-3'
<i>Kcnj10</i>	NM_001039484	5'-CTCCGCTCGCCGCTC-3'	5'-Fam-CAGCCACTTCACCTTCGAGCCAGA-BHQ-3'	5'-ACTGTAAATAGACCTTAGCGACGAC-3'
<i>Nr4a1</i>	NM_010444	5'-GGCCACAGGGAGTGGGA-3'	5'-Fam-CCGGCTGGAGATGCCCTGTATTCAAG-BHQ-3'	5'-CGTTGCTGGTGTTCATATGA-3'
<i>Pdlim4</i>	NM_019417	5'-AGTGACGCGCTGCCG-3'	5'-Fam-ACGGGATCGTGGGAACCATTTGTCA-BHQ-3'	5'-ATGGTAGAGCTTGTCTCTCGCC-3'
<i>Pink1</i>	NM_026880	5'-GAGCGTGGTGGCAATGG-3'	5'-Fam-TGATGGCCCTGAGGTGTCACA-BHQ-3'	5'-CGGCTTTGCTGTAGTCAATTACC-3'
<i>Tnfrsf12a</i>	NM_013749	5'-GCCCGCGGAGAGAAAAAG-3'	5'-Fam-TTACTACCCCATAGAGGAGACTGGTGGAGAG-BHQ-3'	5'-GCCACACCTTGGGACAGC-3'

Table 2
List of primers used to prepare the standards for qRT-PCR

The restriction enzyme (RE) used is indicated in each column preceding the primer sequence. The sequence recognized by the RE is underlined.

Gene Name	Enzyme	Forward primer	Enzyme	Reverse primer
<i>Agr21l</i>	HindIII	5'-CCCAAGCTTGGGATGTGCGAGCTCTATAGGAA-3'	XhoI	5'-CCGCTCGAGCGGCTCATGTCTTGAGTCTTTTGCT-3'
<i>Apod</i>	KpnI	5'-CGGGGTACCCGATGGTGACCATGCTGATGTTCC-3'	EcoRI	5'-CCGGAATTCGGGTACAGGAAGTCCGGGAGTT-3'
<i>Cdkn1a</i>	HindIII	5'-CCCAAGCTTGGGATGTGCAATCCTGGTGATGT-3'	EcoRI	5'-CCGGAATTCGGGTACAGGACTTCACGGGCTGC-3'
<i>Comt</i>	KpnI	5'-GGGGGTACCCGATGCTGTTGGCTGCTGTCTC-3'	EcoRI	5'-CCGGAATTCGGGCTTCAAGGTTTCTCTTGCA-3'
<i>Edg3</i>	EcoRI	5'-CCGGAATTCGGATGGCAACCAACGATGGCA-3'	XhoI	5'-CCGCTCGAGCGGCTCACTTGACAGAGACCCCGT-3'
<i>Fosb</i>	BamHI	5'-GGCGATCCGGGTGAAACCGACAGAGCCTGG-3'	EcoRI	5'-CCGGAATTCGGGTTCTTGCGGGTTTGTGTG-3'
<i>Gadd45b</i>	BamHI	5'-CGCGATCCGATGACCTTGAAGAGCTGGT-3'	EcoRI	5'-CCGGAATTCGGGTGCTCAGCGTTCTCTTA-3'
<i>Gapdh</i>	BamHI	5'-CGCGATCCGATGTTGAAGTCTGGTGTGAA-3'	EcoRI	5'-CCGGAATTCGGGTCTTACTCCTTGGAGCCA-3'
<i>Hbegf</i>	HindIII	5'-CCCAAGCTTGGATGAAGCTGTGCCGTCGGT-3'	EcoRI	5'-CCGGAATTCGGGATAGTCAGGTCTCTCAGTGGG-3'
<i>Hmox1</i>	BamHI	5'-CGCGATCCGCTAGCCAGTCGGTGATGGA-3'	EcoRI	5'-CCGGAATTCGGGTGGGGCCAGTATTGCATT-3'
<i>Kenj10</i>	BamHI	5'-GGCGATCCGCTCCGCTCGCGCTCCTGCC-3'	EcoRI	5'-CCGGAATTCGGGTACAGCTTGCTGATGGCA-3'
<i>Nfkbia</i>	HindIII	5'-CCCAAGCTTGGAGCCATGTTTCAAGCTGCT-3'	XhoI	5'-CCGCTCGAGCGGTTATATGTACAGCGTGGC-3'
<i>Nr4a1</i>	BamHI	5'-CGCGATCCGCTGCTAGAAAGACTGCGGAGC-3'	EcoRI	5'-CCGGAATTCGGGCTTAAAGGCACATGGTG-3'
<i>Pdlim4</i>	BamHI	5'-CCCAAGCTTGGATGACCACTCGGTGACCTG-3'	EcoRI	5'-CCGGAATTCGGGCTCAGACAAAGTTCCACCT-3'
<i>Pink1</i>	KpnI	5'-CGGGGTACCCGATGGCGGTGCGACAGGCACTG-3'	EcoRI	5'-CCGGAATTCGGGTCTGGGCTGCCCTCAGGA-3'
<i>Trifsf12a</i>	BamHI	5'-GGCGATCCGGGCAATCATGGCTCCGGGTG-3'	EcoRI	5'-CCGGAATTCGGGTGAATCACCACTCGCCCCA-3'