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Systems analysis of genetic variation in MPTP neurotoxicity in mice

Byron C. Jones [a,*], Diane B. Miller [b], James P. O'Callaghan [b], Lu Lu [c], Erica L. Unger [d], Gelareh Alam [a], Robert W. Williams [c]

[a] Department of Biobehavioral Health, The Pennsylvania State University, University Park, PA, United States
[b] Centers for Disease Control and Prevention-National Institute for Occupational Safety and Health, Morgantown, WV, United States
[c] University of Tennessee Health Sciences Center, Memphis, TN, United States
[d] Department of Nutritional Sciences, The Pennsylvania State University, University Park, PA, United States

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ABSTRACT

We analyzed genetic variation in severity of neuronal damage using the known dopaminergic neurotoxicant, MPTP, as a prototypical chemical denervation agent. Male mice from ten members of the BXD family of recombinant inbred strains received 12.5 mg/kg MPTP s.c. (vs. saline) and 48 h later brains were taken for multiple related biochemical analyses. Striatal dopamine (DA) and its metabolites, DOPAC and HVA, and serotonin and its metabolite, 5-HIAAA, were analyzed by HPLC. DA turnover was assessed using DOPAC/DA and HVA/DA ratios. Striatal tyrosine hydroxylase (TH), glial fibrilary acidic protein (GFAP), and iron content in ventral midbrain were quantified. All dopamine measures, as well as TH and GFAP, demonstrated wide, genotype-dependent differences in response to MPTP. Serotonin was largely unaffected. Principal components analysis (PC) on difference values, saline minus MPTP, for DA, DOPAC, HVA, and TH, yielded a dominant principal component. The PC trait residuals for each genotype were compared against complementary expression data for striatum of the same strains. Three transcripts representing *Mtap2*, *Lancl 1*, and *Kansl1l* were highly correlated with the PC, as was the difference score, MPTP minus saline for GFAP. This systems approach to the study of environmental neurotoxicants holds promise to define individual genetic differences that contribute to variability in susceptibility to risk factors for diseases such as Parkinson's disease.

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1. Introduction

Exposure to various industrial and agricultural chemicals, especially pesticides, has been implicated as conferring risk for multiple diseases, including neurodegenerative disorders. Several of the substances implicated include rotenone, maneb, paraquat, carbamate and organophosphorus insecticides.

Interactions among multiple risk factors, including gene variants and environmental exposure (e.g., Kitada et al., 2012) are thought to underlie differential vulnerability to neurological disease. Although these factors and their interactions have not been defined, exposure to pesticides—usually associated with rural living—has been implicated as one key environmental cofactor. Epidemiological studies have been equivocal, and some have failed to find consistent associations between pesticide exposure and idiopathic or sporadic Parkinson's disease (sPD) (e.g., Li et al., 2005; van der Mark et al., 2012). If gene–environment interactions (GXE) are fundamental to understanding the etiology of diseases such as sPD, then ascertaining the right set of informative probands becomes problematic. Through the use of reference families of genetically diverse lines of mice, we can address the problems of the complex etiology of sPD and other environmentally related neurodegenerative diseases. This approach addresses the gene–environment interaction framework in which to assess models of disease sensitivity and severity. As proof of concept, the administration of model toxicants to cases with precisely defined genomes has proved to be highly useful (Taylor et al., 1973). One example is recombinant inbred (RI) rodents. RI strains typically are derived from two parental inbred strains by first making an F_1 cross and then inbreeding their offspring by many completely independent full sibling matings for 20 or more generations. This process redistributes (randomly segregates) allelic differences between the parents among a potentially large number of independent but genetically related progeny lines (Peirce et al., 2004). The aim is to develop a genetically diverse group of animals that can be used to model individual differences in genomes seen in genetically segregating populations, such as humans, and then to relate these genomic differences to phenotypic differences

* Corresponding author at: Department of Biobehavioral Health, 315 E. HHD Building, The Pennsylvania State University, University Park, PA 16802, United States. Tel.: +1 814 863 0167; fax: +1 814 863 7525.
E-mail address: bcj1@psu.edu (B.C. Jones).

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observed in nearly any normal or pathological bio-behavioral domain. The use of such a genetic reference population confers several advantages. First, in contrast to single gene mutant studies, the range of the phenotype of interest is revealed and thus some indication of individual differences. Second, the use of multiple strains together with multiple measures in one or more domains provides a powerful tool to examine experimental treatments from a systems biology perspective. Third, when genotyped, the reference population of inbred strains can be queried for polymorphisms that are associated with the phenotypes of interest and may lead to the identification of candidate genes that influence the trait.

As a model neurotoxicant, the proneurotoxin, 1-methyl-4-phenyl-1,2,3,6-tetrahydro-pyridine (MPTP) is a useful denervation tool because it damages the nigro-striatal dopaminergic pathway uniquely and selective antagonists are available to manipulate its neurotoxic effects. This has led to the use of MPTP in animal models of Parkinson's disease (PD); for our purposes, MPTP can be used to reproducibly damage a specific neuronal pathway, absent the influence of other factors, such as blood borne cytokines entering through a damaged blood–brain barrier (BBB) (O'Callaghan et al., 1990). MPTP is readily distributed to the brain without damaging the BBB where it is metabolized by monoamine oxidase-B by glial cells to 1-methyl-4-phenylpyridinium (MPP+) taken up into dopamine (DA) neurons via the DA transporter. MPP+ then disrupts the mitochondrial complex I of the electron transport chain, leading to the accumulation of free radicals which in turn destroy the neuron.

The primary objective of this research was to determine differences in susceptibility on a genetic basis, specifically, based in gene–environment interaction. There is evidence in mice for genetically based differences in MPTP neurotoxicity (Cook et al., 2003) and these authors reported a significantly associated marker (QTL) on chromosome 1 near the telomere. Others have identified QTL related to genetic differences in MPTP toxicity in mice on chromosomes 13 and 15 (Sedelis et al., 2003). In this study, we report genetic differences in the effect of MPTP on multiple neurochemical indices related to dopamine in the caudate–putamen in a random sample of 10 of the BXD family of RI strains derived from C57BL/6J and DBA/2J parental inbred strains. This is the first study of its kind to investigate strain-related differences in MPTP neurotoxicity in a panel of RI strains and using multiple outcome measures to begin to assemble a systems level perspective of MPTP neurotoxicity. Previously mentioned studies report effects on single measures and used F_2 or backcross techniques.

2. Materials and methods

2.1. Animals

Male mice from 10 of the BXD RI strains were used in this study. The animals ranged in age from 2 to 8 months and were reared in the vivarium at UTHSC. Ten days prior to being treated with MPTP, the animals were shipped to the CDC-NIOSH laboratory in Morgantown. The animals had free access to food and water at all times and were maintained on a 12 h:12 h light cycle. All procedures were conducted according to protocols approved by the institutional Animal Care and Use Committee and in accordance with the NRC Guide for the Care and Use of Laboratory Animals (National Academy Press, 1996)

2.2. MPTP and reagents

MPTP–HCl was purchased from Aldrich (Milwaukee, WI, USA). Mouse anti-rat tyrosine hydroxylase (TH) monoclonal antibody was purchased from Sigma (St. Louis, MO) and rabbit anti-rat TH polyclonal antibody was purchased from Calbiochem (San Diego, CA). Antibodies to GFAP are described by O'Callaghan (2002).

2.3. Drug treatment and brain dissection

Following the protocol of O'Callaghan et al. (1990) all animals were injected s.c. with 12.5 mg/kg MPTP, a dose that has been shown previously to cause pronounced effects on dopamine neurochemistry in the caudate–putamen while showing minimal damage to the DA perikarya residing in the SNc (e.g., O'Callaghan et al., 1990). Forty-eight hours after the injection, the animals were killed by decapitation and the brains were removed and dissected freehand to yield the caudate–putamen and ventral midbrain, containing the ventral tegmentum and SNc. For all strains, except BXD32 the numbers of animals treated were 5 each for saline and MPTP. For BXD32 the numbers were 4 each for saline and MPTP.

2.4. Biochemical assays

Using caudate–putamen samples from one side of the brain, dopamine (DA) and its metabolites, dihydroxyphenylacetic acid (DOPAC), homovanillic acid (HVA) and 3 methoxytyramine (3-MT) and serotonin (5-HT) and its metabolite, 5 hydroxyindoleacetic (5-HIAAA) acid were analyzed for content as described below. Values obtained were normalized to tissue wet weight. DA, DOPAC, HVA and other neurotransmitter substances were analyzed by HPLC with electrochemical detection using the following system: tissue homogenates were prepared by sonication (Kontes Micro ultra-sonicator/cell disruptor) on ice using a 30-s pulse in 0.2 M perchloric acid, containing 3,4-dihydroxybenzylamine 1 μM as internal standard. The homogenate was centrifuged at 10,000 × *g* for 15 min, and the resulting supernatant immediately injected using an autosampler described below. The striatum was prepared in a standard volume (0.3 ml) and results were expressed as μg/g original tissue weight. Sample (10 μl) was injected using a temperature controlled (4 °C) Waters 717 Plus Autosampler (Waters, Milford, MA, USA) connected to a Waters 515 HPLC pump. The sample was passed over a reversed-phase C 18 column (Waters Symmetry, 250 × 4.6 mm, 5 μm, 100 Å). Analytes were detected using the Waters 464 pulsed electrochemical detector (range 10 nA, potential 700 mV) connected by means of the Waters bus SAT/IN module to a computer using Millenium Software 32. The mobile phase consisted of 75 mM sodium dihydrogenphosphate, 1.7 mM 1-octanesulfonic acid, 25 μmol ethylendiaminetetraacetic acid and 10% (v/v) acetonitrile. All components were adjusted to a pH of 3.0 with phosphoric acid, pumped at a flow rate of 1 ml/min. Under these conditions the average run time is 30 min with representative retention times (in min) for NE (5.99), 4-dihydroxybenzylamine (DHBA, internal standard, 8.24), DOPAC (8.93), DA (11.28), 5-HIAA (13.57), HVA (19.77), 5-HT (26.1). Quantitation was achieved by the use of the internal standard (10 pmol DHBA per injection) method using daily standard curves of each analyte (0.5–25 pmol per injection). The limit of detection is 0.5 pmol per injection, interassay variation is ±3%. Caudate–putamen samples from the other side of the brain were homogenized in 1% (w/v) SDS heated to 80–90 °C. The concentrations of TH and GFAP in the SDS-total homogenates were analyzed by ELISA (O'Callaghan, 1991, 2002; Sriram et al., 2004). Values obtained were normalized to total homogenate protein assayed by BCA (Smith et al., 1985).

Ventral midbrain iron concentrations were measured according to the modified procedures of Erikson et al. (1997). Briefly, the ventral midbrain was dissected as described by Boone et al. (2007)

weighed and combined with 200 μL of ultrapure nitric acid (OmiTrace®, EM Science, NXO407-1) in a 0.5 mL polypropylene micro-centrifuge tube. Brain regions were digested for 48 h in a 60 °C sand bath and then re-suspended to 400 μL with nanopure water. Each sample was further diluted 1:50 (1:100 for ventral midbrain) with 0.2% ultrapure nitric acid and immediately analyzed for iron by graphite furnace atomic absorption spectrophotometry (Perkin Elmer AAnalyst 600, Perkin Elmer, Norwalk, CT). Standards were prepared by diluting a Perkin Elmer iron standard (PE# N9300126) in 0.2% ultrapure nitric acid and blanks prepared with digesting and diluting reagents to control for possible contamination.

2.5. Data analysis

Main and interaction effects for strain and treatment (MPTP vs. saline) were evaluated by analysis of variance (ANOVA) for a 2 between-subjects variables experiment. Post-hoc pairwise comparisons between saline and MPTP for each strain were made using the Bonferroni t test with α set at 0.05, two tailed. Pairwise comparisons for strain differences under saline treatment for each phenotype were made using the Tukey HSD test.

3. Results

3.1. Effects of MPTP on tyrosine hydroxylase in caudate–putamen

For TH we observed large strain differences in abundance, both in control and in response to MPTP (Fig. 1). Strains 29 and 84 showed the lowest basal levels while the highest was found in strain 9. Strains 29 and 62 were nearly refractory to this dose of MPTP, while strains BXD40, BXD48 and BXD60 showed dramatic reductions in TH abundance.

3.2. Effects of MPTP on dopamine neurochemistry in caudate–putamen

Large and significant strain differences were observed for the basal levels of DA as well as the effect of MPTP (Fig. 2) with strain 29 showing the lowest and strain 9 the highest level ($p < 0.01$).

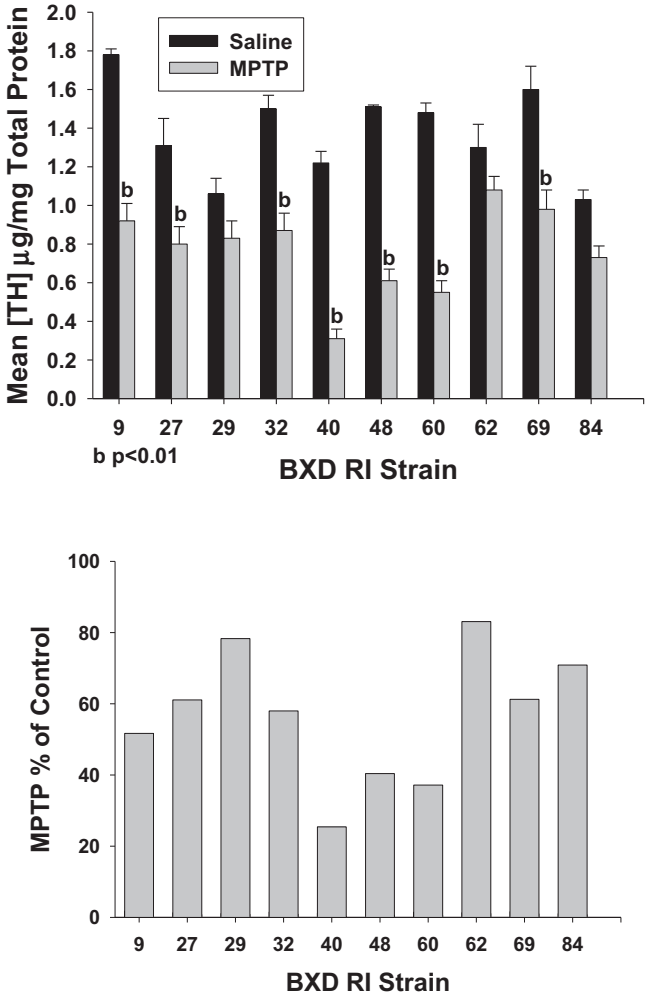

Fig. 1. Effect of 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) on concentration of tyrosine hydroxylase (TH) in the caudate–putamen of 10 BXD recombinant inbred mouse strains. Male mice were injected with 12.5 mg/kg of the pro-neurotoxin, MPTP (vs. saline) and sacrificed 48 h later. TH levels were determined by ELISA. Experimental and control values (upper panel), normalized to total protein, are expressed as means ± s.e.m. The lower panel presents the effect of MPTP expressed as % control. ANOVA revealed significant effects for strain, MPTP and their interaction ($F_{9,78} = 2.95$; $F_{1,78} = 12.91$; $F_{9,78} = 1.74$, respectively; all $p < 0.001$).

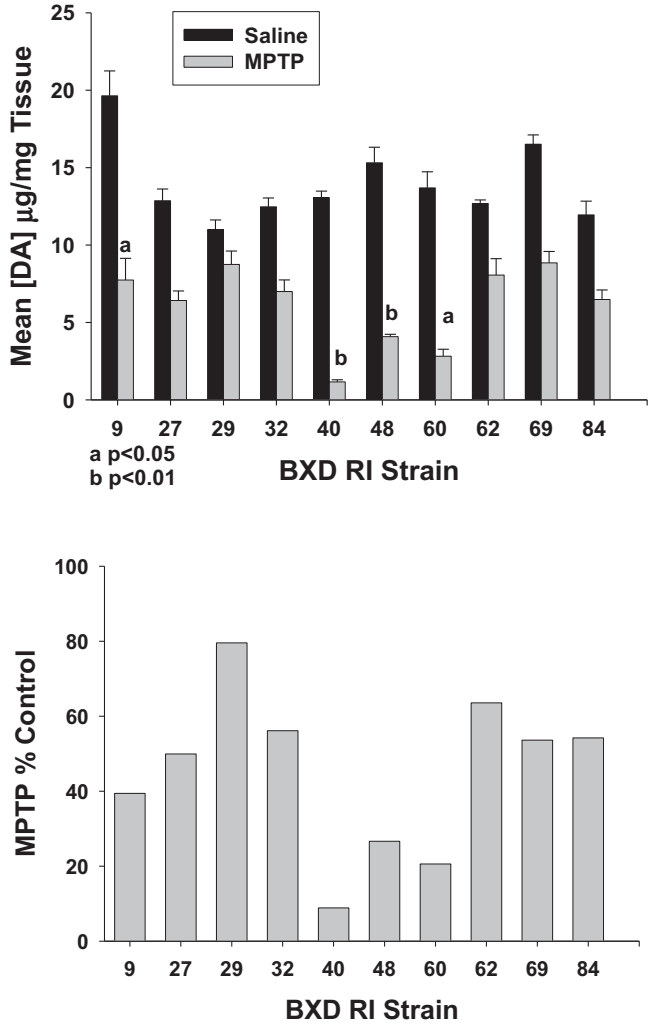

Fig. 2. Effect of 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) on dopamine (DA) concentration in the caudate–putamen in 10 BXD recombinant inbred mouse strains. Male mice were injected with 12.5 mg/kg of the pro-neurotoxin, MPTP (vs. saline) and sacrificed 48 h later. DA was assayed from tissue homogenates by HPLC. Experimental and control values (upper panel), normalized to tissue weights, are expressed as means ± s.e.m. The lower panel presents the effect of MPTP expressed as % control. ANOVA revealed a significant main effect of strain, MPTP and a significant interaction ($F_{9,77} = 11.25$; $F_{1,77} = 445.61$; $F_{9,77} = 9.05$, respectively; all $p < 0.001$).

Strains BXD9, BXD40 and BXD60 showed significant decreases at $p < 0.05$ while for strain BXD48, the decrease was significant at $p < 0.01$.

The DA metabolites, DOPAC (Fig. 3), HVA (Fig. 4), and 3-MT (Fig. 5) were also significantly affected by genotype and MPTP treatment. Strains BXD9, BXD27, BXD29, BXD60 and BXD 69 displayed nearly identical basal levels of DOPAC while the lowest was found in BXD48 and BXD94. The differences among the genotypes, however, did not reach statistical significance. In strains BXD9, BXD27, BXD29, BXD40, BXD48 and BXD60 MPTP treatment produced significant decreases in this metabolite ($p < 0.01$, all) while for strains BXD32, BXD62, BXD69 and BXD84 the decreases observed did not reach significance.

Genotype and treatment also affected HVA levels in striatum (Fig. 4). For this measure, we found the highest basal level in strains BXD9, BXD40, BXD48, BXD69 and the lowest in BXD32 (BXD69 vs. BXD32, $p < 0.01$). Following MPTP treatment significant decreases were observed for BXD9, BXD40, ($p < 0.01$), BXD48, BXD60 and BXD69 ($p < 0.05$) but not BXD27, BXD29, BXD32, BXD62 and BXD84.

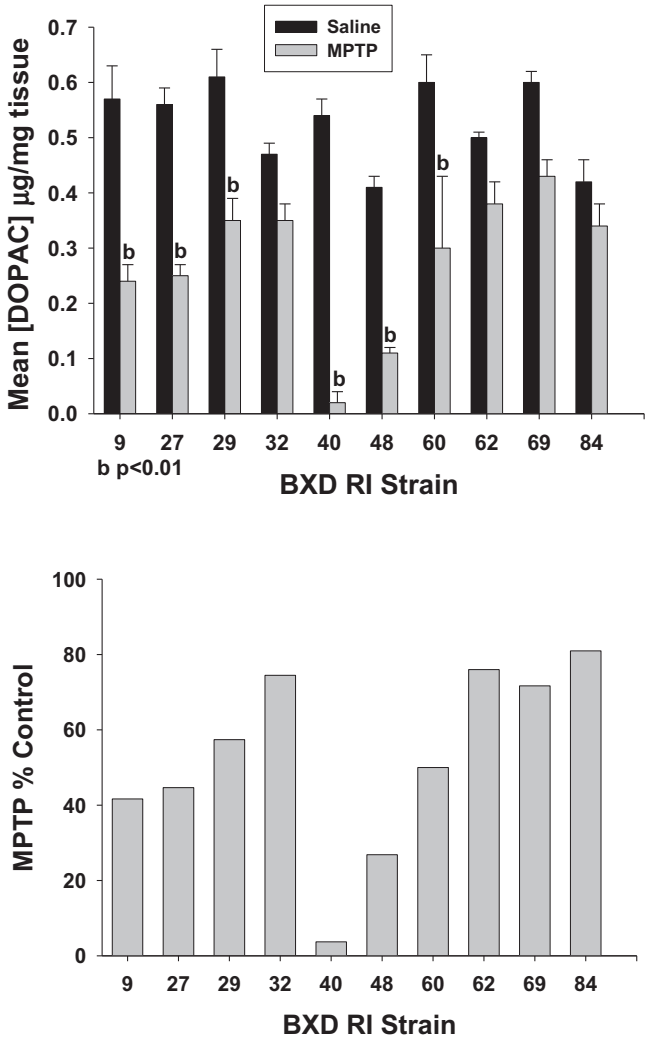

Fig. 3. Effect of 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) on dihydroxyphenylacetic acid (DOPAC) concentration in the caudate–putamen in 10 BXD recombinant inbred mouse strains. Male mice were injected with 12.5 mg/kg of the pro-neurotoxin, MPTP (vs. saline) and sacrificed 48 h later. DOPAC was assayed from tissue homogenates by HPLC. Experimental and control values (upper panel), normalized to tissue weights, are expressed as means ± s.e.m. The lower panel presents the effect of MPTP expressed as % control. ANOVA confirmed significant main and interaction effects for Strain and MPTP ($F_{9,76} = 6.45$; $F_{1,76} = 148.54$; $F_{9,76} = 4.21$; respectively; all $p < 0.001$).

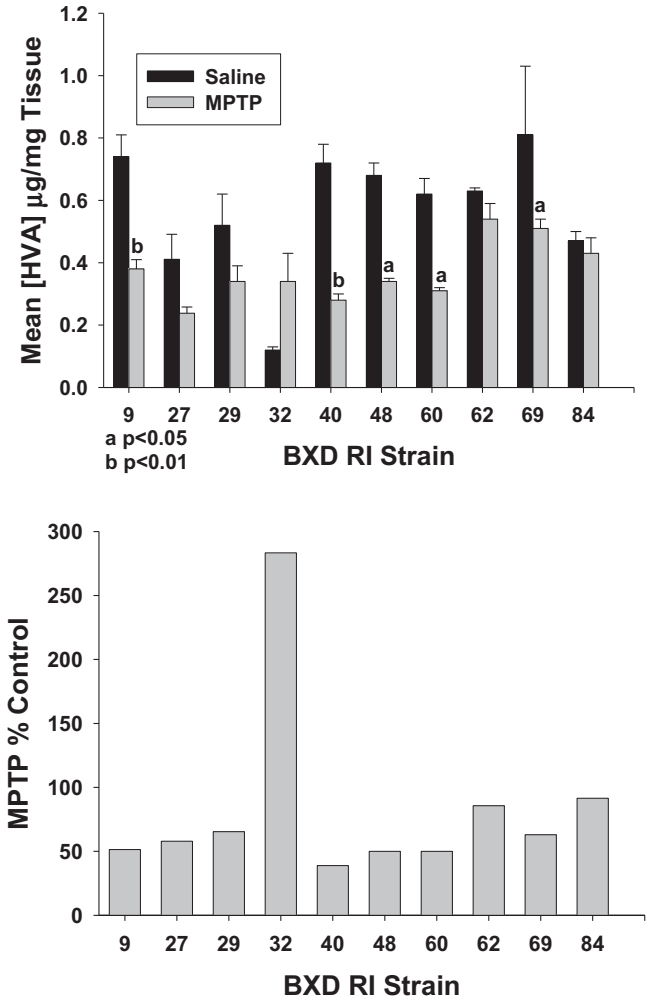

Fig. 4. Effect of 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) on homovanillic acid (HVA) concentration in the caudate–putamen in 10 BXD recombinant inbred mouse strains. Male mice were injected with 12.5 mg/kg of the pro-neurotoxin, MPTP (vs. saline) and sacrificed 48 h later. HVA was assayed from tissue homogenates by HPLC. Experimental and control values (upper panel), normalized to tissue weights, are expressed as means ± s.e.m. The lower panel presents the effect of MPTP expressed as % control. ANOVA indicated significant main and interaction effects for Strain and MPTP ($F_{9,77} = 5.80$; $F_{1,77} = 41.76$; $F_{9,77} = 3.48$, respectively; all $p < 0.01$).

Striatal levels of 3-MT were significantly affected by genotype and treatment but no interaction was detected (Fig. 5). As with DOPAC, there were no pairwise differences among the strain means. Furthermore, pairwise comparison showed that only strain BXD40 showed a significant reduction ($p < 0.01$) by MPTP of this DA metabolite.

Dopamine turnover ratios were also impacted by genotype and treatment. The basal level of DOPAC/DA turnover ratios (Fig. 6), an index reflecting primarily presynaptic processes, was affected by strain with BXD29 showing the highest and BXD 9 and BXD49 the lowest levels ($p < 0.01$ BXD29 vs. BXD49). MPTP decreased this index in BXD40 only ($p < 0.05$).

HVA/DA (Fig. 7) is thought to reflect primarily postsynaptic processes with BXD32 showing an extremely low basal level. None of the pairwise comparisons for basal levels reached significance. In general, MPTP treatment non-significantly increased this index in most of the strains but it was profoundly and significantly increased (~4.5-fold greater than saline control) in BXD40.

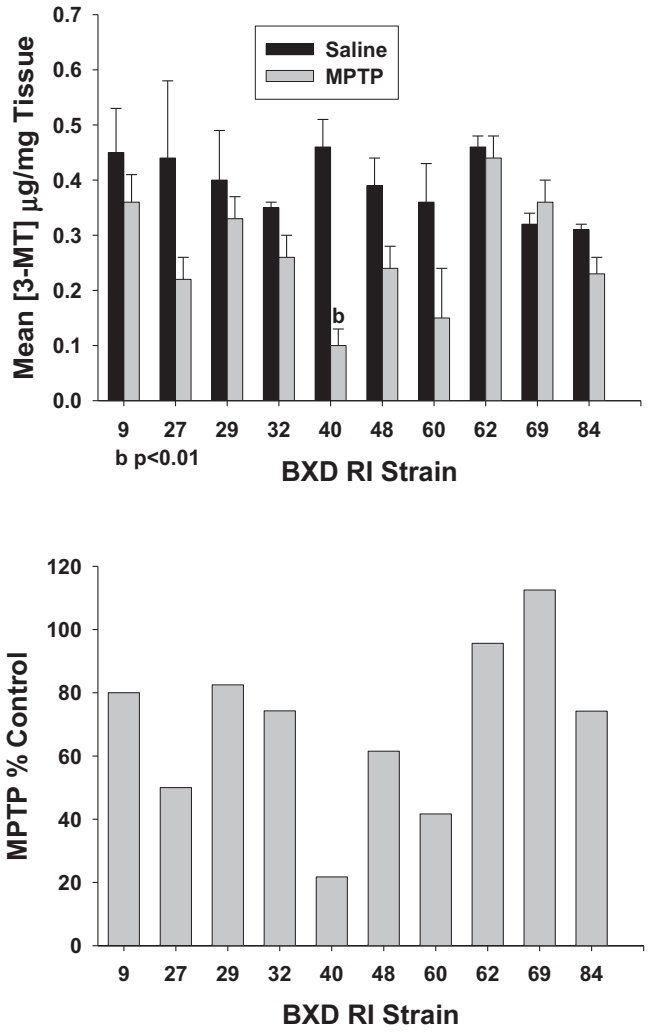

Fig. 5. Effect of 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) on 3-methoxytyramine (3-MT) concentration in the caudate–putamen in 10 BXD recombinant inbred mouse strains. Male mice were injected with 12.5 mg/kg of the pro-neurotoxin, MPTP (vs. saline) and sacrificed 48 h later. 3-MT was assayed from tissue homogenates by HPLC. Experimental and control values (upper panel), normalized to tissue weights, are expressed as means ± s.e.m. The lower panel presents the effect of MPTP expressed as % control. ANOVA showed significant main effects for Strain and MPTP but no interaction ($F_{9,77} = 2.27$, $p < 0.03$; $F_{1,77} = 21.90$, $p < 0.0001$; $F_{9,77} = 1.90$, $p < 0.07$, respectively).

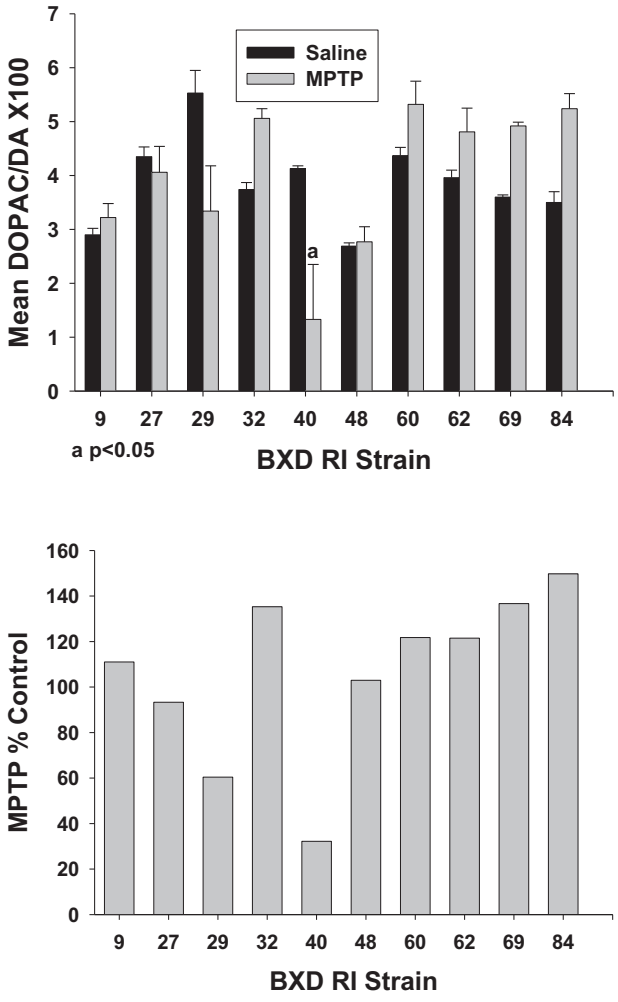

Fig. 6. Effect of 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) dopamine turnover as measured by DOPAC/DA in the caudate–putamen in 10 BXD recombinant inbred mouse strains. Male mice were injected with 12.5 mg/kg of the pro-neurotoxin, MPTP (vs. saline) and sacrificed 48 h later. Experimental and control values (upper panel), normalized to tissue weights, are expressed as means ± s.e.m. The lower panel presents the effect of MPTP expressed as % control. ANOVA revealed no effect of strain on this measure of turnover but a significant effect of strain on the effect of MPTP as well as the interaction between strain and MPTP ($F_{1,77} = <1$; $F_{9,77} = 7.74$, $p < 0.001$; $F_{9,77} = 7.13$, $p < 0.001$, respectively).

3.3. Effects of MPTP on GFAP in caudate–putamen

Genotype and MPTP treatment affected the level of striatal GFAP, an intermediate filament protein and astrocyte marker (Fig. 8), with the lowest basal level found in BXD32 and BXD69 and the highest in BXD9 ($p < 0.01$, BXD9 vs. BXD69). MPTP treatment markedly and significantly increased this protein in BXD9, BXD27, BXD40, BXD48, and BXD69 ($p < 0.01$ for all).

3.4. Effects of MPTP on iron accumulation in ventral midbrain (VMB)

The accumulation of iron in the VMB was affected by strain but not by MPTP treatment (Fig. 9). Only BXD40 showed a significant ($p < 0.01$) increase in iron accumulation (nearly a 40% increase).

3.5. Effects of MPTP on serotonin (5-HT) neurochemistry in caudate–putamen

Large and significant strain differences were observed among basal levels of 5-HT (Fig. 10). BXD48 showed the highest concentration and BXD27 showed the lowest ($p < 0.01$). Only two strains—BXD32 and BXD62—showed significant reductions in 5-HT following MPTP treatment ($p < 0.01$ for both).

3.6. Systems genetic analysis of MPTP effects and correlation with gene expression in the striatum

We combined the difference scores, saline minus MPTP on DA, DOPAC, HVA, and TH (all highly correlated) and used the strain residuals of the first principal component as our index for MPTP neurotoxicity. We then performed a correlation analysis with several large whole transcriptome expression data sets for the same BXD strains (Rosen et al., 2009). These data (accession number 285) are available on www.GeneNetwork.org. The data are from the HQF BXD Striatum Illumina Mouse-6.1 November 2007 Rank Invariant Data Set and corrected (December 2010) for a batch effect due to the hybridization of different strains on different dates. Our index showed high, statistically significant correlations with transcript abundance for many transcripts. Our interest however is in those transcripts with expression that is correlated

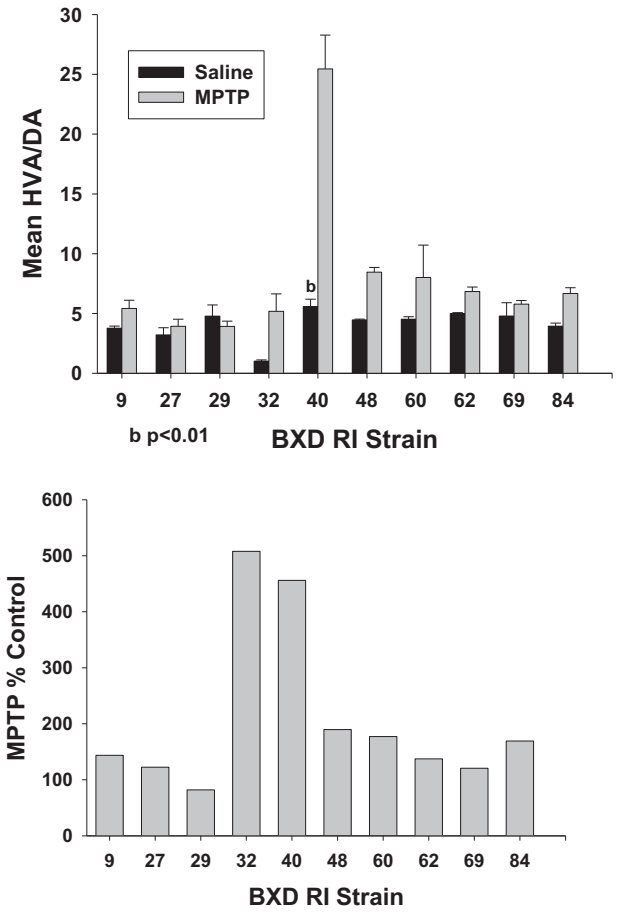

Fig. 7. Effect of 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) dopamine turnover as measured by HVA/DA in the caudate–putamen in 10 BXD recombinant inbred mouse strains. Male mice were injected with 12.5 mg/kg of the pro-neurotoxin, MPTP (vs. saline) and sacrificed 48 h later. Experimental and control values (upper panel), normalized to tissue weights, are expressed as means ± s.e.m. The lower panel presents the effect of MPTP expressed as % control. ANOVA indicated a significant effect of strain and MPTP treatment on this measure of turnover as well as an interaction ($F_{9,77}$ = 21.94; $F_{1,77}$ = 65.13; $F_{9,77}$ = 15.44, respectively; all $p < 0.001$).

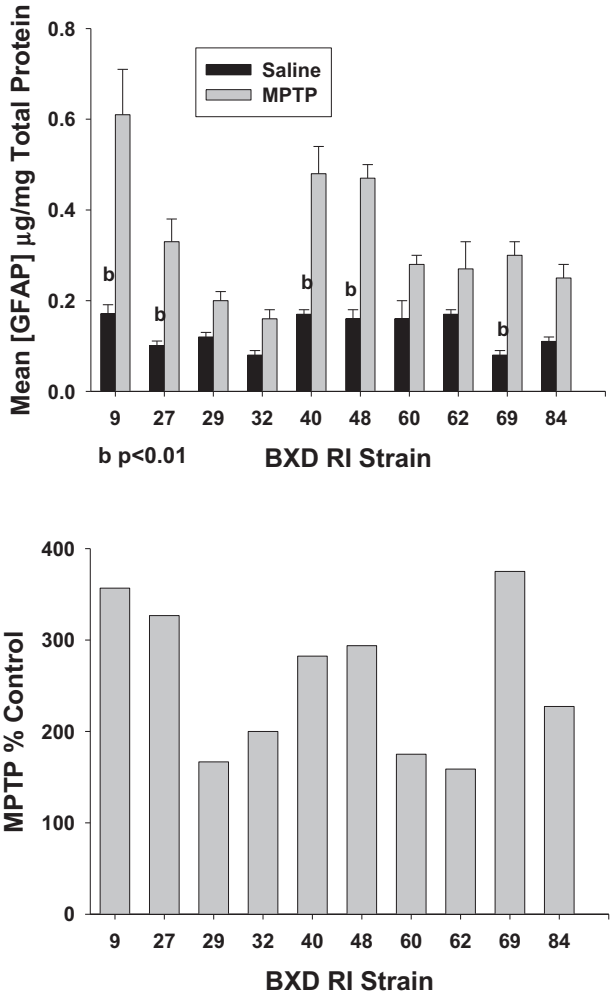

Fig. 8. Effect of 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) on the concentration of glial fibrilary acidic protein (GFAP) in the caudate–putamen of 10 BXD recombinant inbred mouse strains. Male mice were injected with 12.5 mg/kg of the pro-neurotoxin, MPTP (vs. saline) and sacrificed 48 h later. GFAP levels in the caudate–putamen were determined by ELISA. Experimental and control values (upper panel), normalized to total protein, are expressed as means ± s.e.m. The lower panel presents the effect of MPTP expressed as % control. ANOVA revealed significant main effects of strain, MPTP treatment and their interaction ($F_{9,78}$ = 9.76; $F_{1,78}$ = 145.80; $F_{9,78}$ = 5.08, respectively; all $p < 0.001$).

with our index and with each other. Transcripts generated from three genes met both criteria and include *Mtap2* (microtubule associated protein 2), *Lancl* (lantibiotic synthetase component C-like 1 (bacterial) 1) and *Kansl1l* (KAT8 regulatory NSL complex subunit 1-like). The systems diagram is presented in Fig. 11 and the values for the correlations are presented in Table 1.

4. Discussion

This is the first study to report the neurotoxic effects of MPTP in a panel of inbred mouse strains. MPTP at a dose of 12.5 mg/kg (s.c.) produced large variations in multiple indices of neurotoxicity across the 10 BXD RI strains. Moreover, the distribution of the strain differences in all measures is continuous, indicating the influence of multiple genes. MPTP neurotoxicity, thus, is considered to be a complex trait.

As MPTP is a known dopaminergic neurotoxicant it was to be expected for dopamine neurochemistry to be affected to a far greater degree overall than was serotonin. Interestingly, in strain BXD40, MPTP produced the greatest loss of DA from the striatum and was the only strain to show a significant increase of iron in the ventral midbrain. Iron is considered to be a risk factor in sPD *per se*, (Berg et al., 2001) and recently we showed that it co-operates with paraquat in neurotoxicity (Yin et al., 2011). This strain is also quite sensitive to disruption in iron regulation as a result of being fed an iron-poor diet (Jellen et al., 2012). That this strain is also among the most susceptible to MPTP toxicity and exactly how iron and MPTP toxicity in this strain are related remains to be seen. One hypothesis is that for BXD40, the 12.5 mg/kg dose does indeed damage DA neurons in the SNc by way of iron homeostasis dysregulation. Previously, we showed that paraquat neurotoxicity is likely related to its dysregulation of iron homeostasis in the ventral midbrain (Yin et al., 2011). MPTP may produce the same effect, with individual differences. If true, then higher doses of MPTP should involve SNc damage and increase iron influx in more strains.

This study was designed as proof-of-concept to show that not all inbred mouse strains (hence humans) are equally susceptible to MPTP neurotoxicity, and likely other toxicants as well. This study also demonstrates the power of systems genetic and systems biology analysis. We did not measure the production of MPP+ and the degree to which individual differences in MPTP neurotoxicity is related to differences in the production of this neurotoxic metabolite will need to be addressed in future studies.

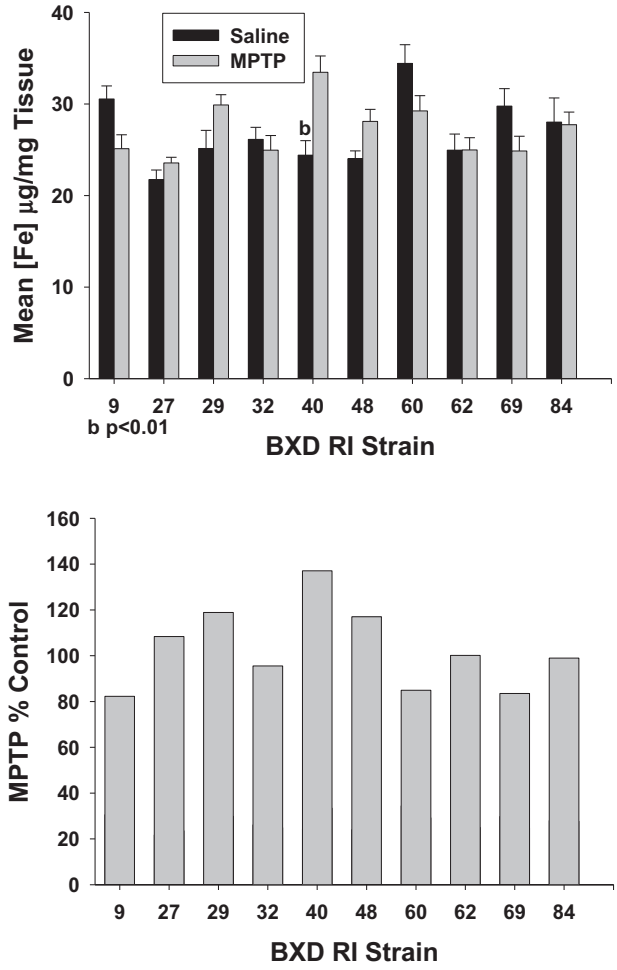

Fig. 9. Effect of 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) on iron concentration in the ventral midbrain in 10 BXD recombinant inbred mouse strains. Male mice were injected with 12.5 mg/kg of the pro-neurotoxin, MPTP (vs. saline) and sacrificed 48 h later. Atomic absorption spectroscopy was used to determine iron concentration from tissue homogenates. Experimental and control values (upper panel), normalized to tissue weight, are expressed as means ± s.e.m. The lower panel presents the effect of MPTP expressed as % control. ANOVA indicated a significant effect of strain ($F_{9,77} = 4.92$, $p < 0.001$) and interaction between strain and MPTP treatment ($F_{9,77} = 4.55$, $p < 0.001$) but not MPTP treatment ($F_{1,77} < 1$).

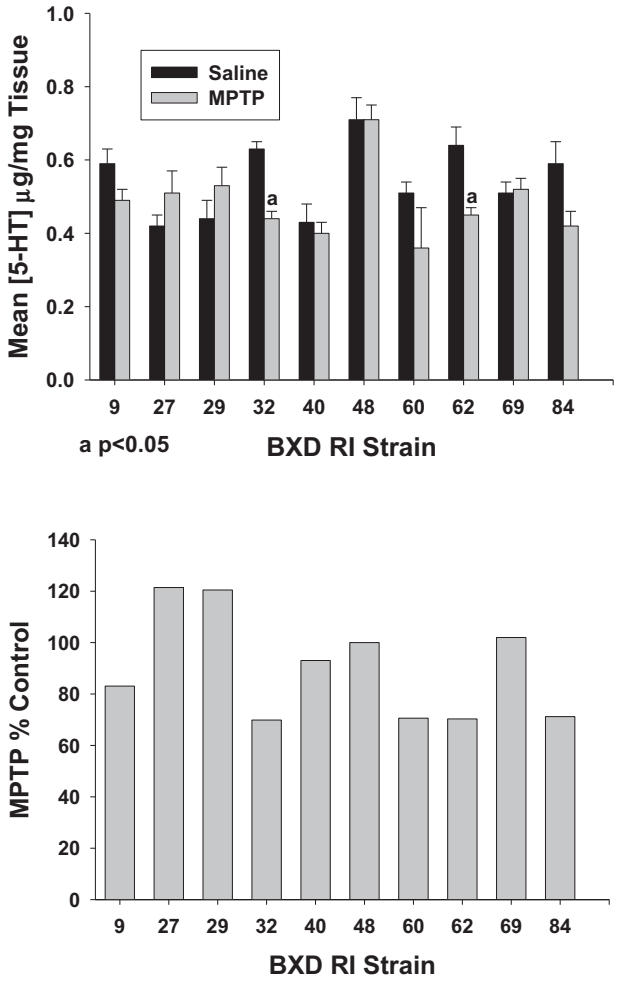

Fig. 10. Effect of 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) on serotonin (5-HT) concentration in the caudate–putamen in 10 BXD recombinant inbred mouse strains. Male mice were injected with 12.5 mg/kg of the pro-neurotoxin, MPTP (vs. saline) and sacrificed 48 h later. DA was assayed from tissue homogenates by HPLC. Experimental and control values (upper panel), normalized to tissue weights, are expressed as means ± s.e.m. The lower panel presents the effect of MPTP expressed as % control. ANOVA revealed a significant main effect of strain, MPTP and a significant interaction ($F_{9,77} = 6.15$, $p < 0.001$; $F_{1,77} = 9.64$, $p < 0.005$; $F_{9,77} = 2.63$, $p < 0.02$, respectively).

Now that we have demonstrated large genetically controlled differences in sensitivity to MPTP, the next step is to find gene variants that underlie these differences. In this prelude, we have used only 10 of ~150 BXD lines. QTL mapping with only 10 strains is risky; nevertheless, when we performed PCA on mean differences, saline-MPTP on TH, DA, DOPAC and HVA and mapped the first principal component, we obtained an intriguing and nominally significant QTL (LOD = 4.3) at ~60 ± 10 Mb on chromosome 1. Interestingly, the three genes that we identified as correlated with our measure of dopamine neurotoxicity are all *cis*-regulated with eQTL at the same locus (~66 Mb on chromosome 1) as the dopamine-related QTL. This QTL will require verification by testing many more of the BXD strains. QTL analysis coupled with gene expression data is a very powerful technique for the nomination of candidate genes. In order to identify genes and mechanisms underlying individual differences in MPTP neurotoxicity, gene expression studies would have to be conducted in the SNc and caudate–putamen. The SNc is where TH, dopamine transporter and dopamine autoreceptors are synthesized and transported to the caudate–putamen. Gene expression studies in the latter tissue could help us understand the "collateral damage" and its contribution to sPD by an impaired DA transmission system. What about the genes that we identified whose expression correlates highly with our index of MPTP neurotoxicity? *Lancl1* binds glutathione and is important in oxidative stress and related diseases (Zhong et al., 2012). *Mtap2* is known to bind to TWIK-related potassium channels (Sandoz et al., 2008) and may be involved in maintaining the integrity of dendritic microtubules, thus playing an important role in signal transduction (as reviewed by Goldstein and Yang, 2000). *Kansl1l* is also known as human *KAT8* K(lysine) acetyltransferase 8 and acetylates histones in gene regulation. Presumably, the function will be the same in mouse. The association between MPTP neurotoxicity and expression of these genes presents new information on possible mechanisms underlying host susceptibility characteristics and sets the stage for more extensive study in a large panel of BXD RI (or other) mouse strains.

Finally, the systems analysis revealed important interrelationships among our phenotypes and the expression of genes that

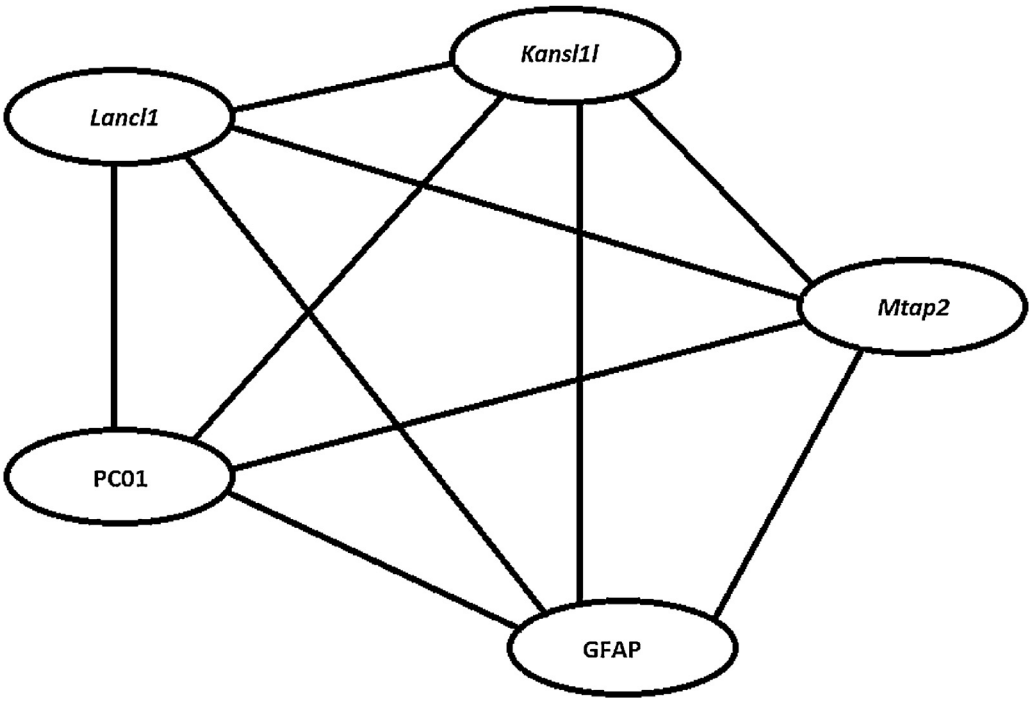

Fig. 11. System network graph for striatal GFAP following MPTP (label 15159), MPTP neurotoxicity index (PC01), and transcript abundance for genes *Kansl1l*, *Lancl1* and *Mtap2*. This is a graphical representation of the data presented in Table 1.

Table 1
Correlation matrix for the graphical representation of the system analysis presented in Fig. 11. Correlations presented are Pearson *r*.

	GFAP	Kansl1l	Lancl1	Mtap2
Kansl1l	$0.80^{7,a}$			
Lancl1	$0.78^{7,a}$	$0.93^{57,b}$		
Mtap2	$0.75^{7,a}$	$0.94^{57,b}$	$0.92^{57,b}$	
PC	$-0.81^{8,a}$	$-0.93^{57,b}$	$-0.87^{7,b}$	$-0.87^{7,a}$

n = df
[a] $p < 0.05$.
[b] $p < 0.01$.

likely underlie individual differences in sensitivity to MPTP, and perhaps other toxicants, neurotoxicity.

5. Conclusion

We have shown wide, genetic variation in response to MPTP among 10 BXD genotypes across multiple MPTP-related phenotypes. Multivariate analysis has shown the rich landscape of associations among these indices and increasing the number of strains and doses will prove invaluable in elucidating the gene–environment underpinnings of sPD and will pave the way for similar study of similar diseases.

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Conflict of interest statement

None.

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