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Differential Contribution of Lipoxygenase Isozymes to Nigrostriatal Vulnerability

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Abstract

The 5- and 12/15- lipoxygenase (LOX) isozymes have been implicated to contribute to disease development in CNS disorders such as Alzheimer's disease. These LOX isozymes are distinct in function, with differential effects on neuroinflammation, and the impact of the distinct isozymes in the pathogenesis of Parkinson's disease (PD) has not as yet been evaluated. To determine whether the isozymes contribute differently to nigrostriatal vulnerability, the effects of 5- and 12/15-LOX deficiency on dopaminergic tone under na ve and toxicant-challenged conditions were tested. In na ve mice deficient in 5-LOX expression, a modest but significant reduction (18.0% reduction vs. wildtype (WT)) in striatal dopamine (DA) was detected (n=6-8 per genotype). A concomitant decline in striatal tyrosine hydroxylase (TH) enzyme was also revealed in null 5-LOX vs. WT mice (26.2%); however, no changes in levels of DA or TH immunoreactivity were observed in null 12/15-LOX vs. WT mice. When challenged with the selective dopaminergic toxin, 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP), WT mice showed marked reduction in DA (31.9%) and robust astrocytic and microglial activation as compared to saline-treated animals. In contrast, null 5-LOX littermates demonstrated no significant striatal DA depletion or astrogliosis (as noted by Western blot analyses for GFAP immunoreactivity). In na ve null 12/15-LOX mice, no significant change in striatal DA values was observed compared to WT, and following MPTP treatment, the transgenics revealed striatal DA reduction similar to the challenged WT mice. Taken together, these data provide the first evidence that: (i) LOX isozymes are involved in the maintenance of normal dopaminergic function in the striatum, and (ii) the 5- and 12/15-LOX isozymes contribute differentially to striatal vulnerability in response to neurotoxicant challenge.

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Keywords

Parkinson's disease; nigrostriatal; dopamine; lipoxygenase; MPTP; inflammation

1. Introduction

Parkinson's disease (PD) is a progressive CNS disorder characterized behaviorally by motor impairment and neurochemically by a loss of nigrostriatal dopaminergic tone. Fundamental processes thought to underlie ongoing degeneration in PD include oxidative damage, mitochondrial dysfunction, protein clearance abnormalities and protein misfolding (Imai and Lu, 2011; Plowey and Chu, 2011; Breydo et al., 2012; McCoy and Cookson, 2012). Inflammation has been implicated to contribute to PD pathogenesis as revealed by epidemiological and pathological studies as well as experimental modeling but whether these are primary or secondary processes is not well understood (Hunot and Hirsch, 2003;Tansey et al., 2008; Ouchi et al., 2009; Ton et al., 2012). Glial involvement (i.e. astrogliosis and microglial activation) in cell death and dysfunction have been demonstrated to play a key role (Ton et al., 2006; Tansey et al., 2008; Tansey and Goldberg, 2010; Ton et al., 2012), and eicosanoids, the collective name for products of arachidonic acid (AA) metabolism, have been widely studied in mediating activation at a cellular level (Minghetti, 2004; Farooqui and Horrocks, 2006; Farooqui et al., 2007; Adibhatla and Hatcher, 2008). The lipoxygenases (LOX) are a family of distinct isozymes that catalyze oxidation of AA and consequently contribute to toxicity through the generation of free reactive oxygen species (ROS), toxic lipid hydroperoxides and potent cytokines (Serhan and Samuelsson, 1988; Zaleska and Wilson, 1989; Simonian and Coyle, 1996; Manev, 2000; Sugaya et al., 2000; Mytilineou et al., 2002; Parkinson, 2006; Serhan, 2006; Yang et al., 2010). The LOX enzymes have been shown to play a central role in pathogenesis in a variety of disease states including diabetes, asthma, cancer, heart disease, and most recently, neurodegenerative conditions (Serhan and Samuelsson, 1988; Parkinson, 2006; Serhan, 2006; Cheng et al., 2008; van Leyen et al., 2008; Weaver et al., 2012; Yeung et al., 2012) including Alzheimer's disease (AD) (Pratico et al., 2004; Manev and Manev, 2006; Listi et al., 2010; Yang et al., 2010; Chu and Pratico, 2011; Manev et al., 2011; Puccio et al., 2011) and Creutzfeldt-Jakob disease (Stewart et al., 2001; Phillis et al., 2006). Despite that LOX isozymes are well-established in lipid-mediated cellular responses, understanding of their role(s) in the nigrostriatal pathway represents a gap in knowledge.

All LOX isozymes are expressed in the brain (Bendani et al., 1995; Manev et al., 2000a, 2001; Qu et al., 2000; Uz et al., 2001a; Uz et al., 2001b). including the ventral midbrain which contains the nigrostriatal neuronal cell bodies affected in PD (Mytilineou et al., 1999). The expression of LOX is enhanced in the brain with aging and in models of neurodegeneration and inflammation (Uz et al., 1998; Manev et al., 2000b; Qu et al., 2000; Firuzi et al., 2008; Basselin et al., 2010). Recent studies related to CNS disease have focused primarily on the 5- and 12/15-LOX isozymes (Chu and Pratico, 2011; Hashimoto, 2011; Manev et al., 2011; Puccio et al., 2011; Chu et al., 2012a,b). The LOX isozymes catalyze stereospecific modification of AA at either carbon position 5, 12 or 15 or both 12 and 15, and the site of molecular oxygen insertion correspondingly defines the isozyme (i.e. 5-, 12-,

15- or a dual specificity 12/15-LOX, respectively). The reaction product of AA oxidation is hydroperoxyeicosatetraenoic acid (HPETE); this unstable compound is subsequently reduced by glutathione peroxidase to the more stable hydroxyeicosatetraenoic acid (HETE) (Brash, 1999). While 5-, 12-, 15- and 12/15-LOX can generate HETE forms, the distinct products have selective downstream effects. For example, 12-HETE, but not 5-HETE, antagonizes the activity of the peroxisome proliferator activator receptor gamma (PPARγ) to promote glial cell activation (Lopez-Parra et al., 2005; Limor et al., 2008). Interestingly, 5-HETE is also a substrate for the 5-LOX isozyme, catalyzing the formation of the epoxide, leukotriene A_4, which is subsequently converted to leukotriene B4 (LTB_4). LTB_4 is a highly potent inflammatory factor and promotes enhanced expression of IL-12, IL-6 and TNFα (Phillis et al., 2006; Tassoni et al., 2008).

Although inhibition of 5- and 12/15-LOX has been shown to protect against the detrimental outcomes in disease models of stroke and AD (Klegeris and McGeer, 2002; Wang et al., 2006; Jin et al., 2008; Sobrado et al., 2009; Cui et al., 2010; Tu et al., 2010), the role of these isozymes in the nigrostriatal pathway has not been fully evaluated. The development and availability of mouse models with targeted gene deletions provide critical tools to better understand the role of LOX in degeneration related to PD. To determine if the 5- and 12/15-LOX isozymes selectively contribute to nigrostriatal vulnerability, the impact of 5- or 12/15-LOX deficiency on striatal dopaminergic tone and injury was tested in mouse models under na ve and toxicant-challenged conditions.

2. Experimental Procedures

2.1 Animals

Transgenic mice deficient in 5-LOX (004155; sex-matched and aged 8 weeks) or 12/15-LOX (002778; males aged 8 weeks) and respective sex-matched strain controls were purchased from The Jackson Laboratory (Bar Harbor, ME). All mice were housed on a 12-h light–dark cycle and given free access to food and drinking water as well as environmental enrichment. All animal procedures and animal care methods were approved by the Institutional Animal Care and Usage Committees for SRI International.

2.1.1 MPTP exposure—The dopaminergic neurotoxin 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) (Sigma, St. Louis, MO) was dissolved in sterile saline. Mice received saline or 15 mg/kg MPTP, i.p., daily for 5 days. All animals were weighed daily during MPTP administration and monitored until 72 hours after the last MPTP exposure. Animals were euthanized by cervical dislocation 7 days following the last MPTP injection.

2.2 Tissue Processing

Midbrain was immersion-fixed in 4% paraformaldehyde overnight, cryoprotected in graded sucrose solutions and frozen at −80°C. Coronal sections were collected on a Leica cryostat (Buffalo Grove, IL) at 40 μm thickness for histology. Striatal tissue was dissected from a forebrain slice (1-2 mm thick) at the level of the anterior commissure and frozen immediately on dry ice. Tissues were sonicated and centrifuged, and homogenate fractions

collected for biochemical analyses by Western blot and neurochemical analyses of dopamine (DA) and metabolite levels.

2.2.1 Neurochemistry—Striatal samples were placed in 1 ml ice-cold 0.4 M perchloric acid, sonicated and centrifuged at 15,000 × *g* for 12 min. The supernatant was collected for assay of DA and its metabolites, dihydroxyphenylacetic acid (DOPAC) and homovanillic acid (HVA) by HPLC with electrochemical detection (Coulochem III detector; Dionex/Thermo Scientific) using a reverse phase C18 column (MD-150; Dionex/Thermo Scientific) (Kilpatrick et al., 1986). The pellet was dried and reconstituted in 0.5N NaOH and briefly sonicated, and total protein determined using the Lowry method (Manning-Bog et al., 2007).

2.2.2 Immunoblotting—Proteins separated by the sample preparation were used for immunoblotting experiments. Following sonication in Tris-EDTA buffer with protease and phosphatase inhibitors (Sigma), samples were centrifuged at 1,000 × *g* for 10 min, the supernatant aspirated, and the pellet reconstituted in sample buffer. The protein concentration was measured by Pierce BCA assay (Thermo Scientific, Rockford, IL) and supernatant proteins separated by SDS-PAGE (12% Tris-glycine, Invitrogen/Life Technologies, Carlsbad, CA) then transferred to nitrocellulose. Blots were blocked in 5% non-fat milk and incubated overnight at 4°C with rabbit anti-tyrosine hydroxylase (TH; Pel-Freez Biologicals, Rogers, AK; and Chemicon/Millipore, Billerica, MA), rabbit anti-*β*-actin (Sigma), and mouse anti-glial acidic fibrillary protein (GFAP; Covance Inc., Princeton, NJ). Next, appropriate peroxidase-conjugated secondary antibodies were applied, and signal visualized following incubation with Pierce chemiluminescent substrate (Thermo Scientific). Blots were placed in plastic sleeves and exposed to CL-XPosure Film (Thermo Scientific), and the immunoreactivity was quantified by Image J software.

2.2.3 Immunohistochemistry—Coronal tissue sections (40 μm) were obtained as described above and stored in cryoprotectant solution (0.01M phosphate buffer, 30% sucrose, 30% ethylene glycol, pH 7.4) at −20°C until further use. The standard protocol that was used to detect primary antibodies has been described previously (Manning-Bog et al., 2003). Incubation times varied depending on the primary and secondary antibodies that were utilized. Antibodies used were rabbit and sheep anti-TH (Pel-Freez and Chemicon/Millipore), rabbit anti- GFAP (Chemicon/Millipore), and rabbit anti-Iba1 (Biocare Medical, Walnut Creek, CA). IgG from the primary species served as the negative control. Immunohistochemistry was either by immunofluorescence with secondary antibodies conjugated to Alexa Fluor 568 (AF568; Invitrogen) or FITC (Jackson ImmunoResearch Laboratories, West Grove, PA), or by 3,3′-diaminobenzidine (DAB) visualization (avidin-biotin system from Vector Laboratories, Burlingame, CA). Immunofluorescent sections were mounted using anti-fade mounting medium with DAPI (Vector Laboratories). DAB sections were lightly counterstained with 0.5% cresyl violet (FD Neurotechnologies, Ellicott City, MD), dehydrated successively in alcohols and xylenes and coverslipped with Permount (Fisher Scientific, Atlanta, GA). Positive immunoreactivity was observed by a light microscope (Eclipse E400; Nikon, Melville, NY) equipped for epi-fluorescence.

2.3 Statistics

All experiments were repeated to ensure reproducibility. Differences among means were analyzed using one-way analysis of variance (ANOVA) when comparing differences between genotype and two-way ANOVA when comparing differences between genotype and toxicant treatment. Tukey's HSD post hoc analysis was employed when differences were observed in ANOVA testing ($p \leq 0.05$).

3. Results

To determine whether LOX isozymes contribute to nigrostriatal dopaminergic function and vulnerability to toxicity, two transgenic mouse lines deficient in 5- or 12/15-LOX were utilized. In striatal homogenates from null 5-LOX mice, a significant reduction (18.0%) in DA values was measured as compared to wildtype littermates (WT; Fig. 1A). No change was detected in the metabolite DOPAC, but levels of HVA were significantly lower (25.7%) in the 5-LOX-deficient vs. WT mice (Fig. 1A). To determine if this effect were specific to the 5-LOX isozyme, or rather a common feature to general LOX deficiency, striatal samples were obtained from null 12/15-LOX vs. WT mice and tested for DA and metabolite levels. No differences in striatal DA or HVA were measured in the animals lacking 12/15-LOX (Fig. 1B). However, a modest decrease was discovered in DOPAC (9.3%) in the 12/15-LOX vs. WT mice (Fig. 1B).

One possibility for the difference in DA values in the null 5-LOX mice is a change in synthesis machinery, such as levels of TH protein, the rate-limiting enzyme in DA synthesis. Consequently, TH protein expression measurement was performed using samples obtained from the other striatal hemisphere. Western blot analyses revealed that the TH level was significantly lower (26.2%) in null 5-LOX vs. WT striatum (Fig. 1C). No difference in TH protein immunoreactivity was observed in striatum between null 12/15-LOX vs. WT littermates (data not shown). These data indicate differential effects on striatal TH expression and DA synthesis and metabolism by the two isozymes.

The transgenic lines were subsequently utilized to address whether LOX isozymes differentially contribute to nigrostriatal vulnerability. Null 5-LOX and WT were exposed to the dopaminergic neurotoxin, MPTP using a subacute paradigm (Manning-Bog et al., 2007). In WT mice, treatment with 15 mg/kg MPTP, i.p., once daily for five consecutive days resulted in a 31.9% reduction in striatal DA values as compared to WT mice injected with saline. In null 5-LOX mice, MPTP treatment did not significantly change DA levels vs. saline-treated animals (Fig. 2A). Similarly, the metabolite HVA was significantly reduced in MPTP-exposed WT mice as compared to saline exposed (8.24 ± 0.79 vs. 10.57 ± 1.31 ng/mg protein, respectively), but MPTP toxicity was not observed in the null 5-LOX transgenics which revealed HVA values equivalent to saline-treated null 5-LOX mice (7.02 ± 1.39 vs. 7.74 ± 1.00 ng/mg protein, respectively) (Fig. 2C). Although no change in striatal DOPAC was measured (Fig. 2B), increased DA turnover (37.6%) was revealed in MPTP vs. saline-injected WT mice whereas no significant difference in turnover was noted in MPTP vs. saline-exposed null 5-LOX transgenics (Fig. 2D).

Null 12/15-LOX and WT littermate mice were also challenged with MPTP. However, with this LOX transgenic line, no significant difference in MPTP-mediated injury was observed (Fig. 3). Striatal DA was depleted by 70.4% in WT mice and 70.5% in 12/15-LOX-deficient littermates (Fig. 3A); similar reductions in DOPAC and HVA were observed (Fig. 3B, C).

To confirm protection from MPTP-induced damage, TH expression was measured using homogenates obtained from the other striatal hemisphere. A 38.0% reduction in TH immunoreactivity, as determined by semi-quantitative Western blot analyses, occurred in WT mice treated with MPTP compared to mice administered saline; however, in 5-LOX-deficient mice, striatal TH immunoreactivity was not significantly different between the saline- and MPTP-challenged groups (Fig. 4A). Representative images of immunofluorescence in the substantia nigra show a dramatic depletion of FITC-labeled TH-positive dopaminergic neurons in the WT MPTP cohort, but not the null 5-LOX MPTP group (Fig. 4B).

A marker for astrocytic activation (i.e. neuroinflammation), GFAP, was also assessed. While little to no difference in striatal GFAP immunoreactivity was detected in WT and 5-LOX-deficient littermates administered saline, a marked increase in GFAP levels was revealed in WT treated with MPTP. This change was absent from the MPTP-challenged null 5-LOX animals (Fig. 5A); GFAP immunoreactivity in this cohort was analogous to mice exposed to saline. Consistent with this finding, more robust labeling by anti-Iba-1 was apparent in nigral microglia in WT than null 5-LOX animals treated with the toxin (Fig. 5B, right panels). Additionally, nigral microglia in the MPTP-treated null 5-LOX cohort demonstrated a ramified appearance with soma similar to the saline-treated groups (Fig. 5B). In contrast, microglia in the WT MPTP group demonstrated morphology indicative of the activated state, more ovoid cell body and less ramified with retracted processes (Kettenmann et al., 2011) (Fig. 5B, top right panel).

4. Discussion

The purpose of this study was to investigate LOX isozyme-specific modifications to the nigrostriatal pathway that may contribute to pathophysiology in PD. While extensively studied for pro- and anti-inflammatory effects in other conditions such as diabetes, cancer, asthma, cardiovascular disorders and most recently AD (Marks et al., 2000; Werz, 2002; Yao et al., 2005; Gubitosi-Klug et al., 2008; Ikonomovic et al., 2008; Planaguma et al., 2008; Liu et al., 2009; Menna et al., 2010; Neilson et al., 2012), LOX enzymes have not been widely explored in the context of PD etiology and progression (Mosca et al., 1996; Li et al., 1997; Mytilineou et al., 1999, 2002; Klegeris and McGeer, 2002; Canals et al., 2003; de Bernardo et al., 2004; Kramer et al., 2004; Scholz et al., 2008; Gupta et al., 2010; Dobrian et al., 2011). Further, although products of distinct LOX-mediated metabolism have differential effects on activation pro- and anti-inflammatory cellular pathways, no study has compared isozyme-specific effects on nigrostriatal dopamine regulation and vulnerability. In summary, these studies show that: (1) the 5- and 12/15- LOX isozymes have distinct effects within the nigrostriatal pathway on DA metabolism and response to neurotoxic insult, and (2) the functional impact of 5-LOX activity changes depending on the health condition (i.e. normal or injured) in the mouse model.

The differential impact of 5- and 12/15-LOX deficiency on dopaminergic neuroprotection in the nigrostriatal pathway following toxicant exposure (Figs. 2-4) is not entirely surprising as isozyme-selective effects have been reported previously with these LOX-deficient transgenic lines (Gubitosi-Klug et al., 2008). Consistent with our findings, in diabetic retinopathy studies, mice lacking 5-LOX demonstrated significantly reduced capillary degeneration as compared to null 12/15-LOX transgenics and WT mice. Furthermore, the histopathology in the diabetic 12/15-LOX genotype was not significantly different from diabetic WT animals (Gubitosi-Klug et al., 2008). In the present study, striatal MPTP toxicity was attenuated in null 5-LOX transgenics as evidenced by higher striatal DA and metabolite values (Fig. 2) and TH immunoreactivities in the striatum (Fig. 4) and substantia nigra (Fig. 5). Neuroprotection was not observed in MPTP-challenged 12/15-LOX mice, however, as these transgenics responded similarly to exposed WT mice (Fig. 3). Taken together, these data demonstrate LOX isozyme-selective effects on vulnerability of the nigrostriatal pathway to injury.

Both 5- and 12-LOX have been previously shown to impact CNS dopaminergic systems, particularly in response to agents that selectively target dopaminergic neurons. The isozymes, for example, affect the behavioral and biochemical responses to the dopamine re-uptake inhibitor cocaine (Kurtuncu et al., 2008, Chen and Manev, 2011). Mice deficient in 5- or 12-LOX exposed to repeated cocaine exposures do not develop locomotor sensitization as is noted in wildtype control mice (Kurtuncu et al., 2008). This effect is likely attributable to enhanced site-specific GluR1 phosphorylation which occurs in striatum of 5-LOX-deficient mice (Chen and Manev, 2011), that impacts membrane insertion of the receptor and its activity; similar alterations have been observed using a pharmacological model of 5-LOX inhibition, minocycline (Imbesi et al., 2008). Thus, it is possible that products of the isozyme's activity, through interactions with AMPA receptors, contribute to a 5-LOX-mediated response to dopaminergic agents.

Although LOX-isozyme activity can promote damage via oxidative stress-producing events such as lipid hydroperoxide formation and glutathione depletion (Roy et al., 1994, 1995; Pratico et al., 2004; Sun et al., 2012), LOX toxicity is most commonly associated with eicosanoid production that elicits cytotoxic inflammatory responses (Manev et al., 2011). Indeed, markers of inflammation, including both astrocytic and microglial activation were depressed in the toxicant-challenged null 5-LOX mice as compared to WT littermates (Fig. 5). The 5-LOX product, 5-HETE, is converted to LTB_4, a highly potent endogenous leukocyte attractant that promotes enhanced expression of IL-12, IL-6 and TNFα (Phillis et al., 2006; Tassoni et al., 2008). While our findings of glial activation support the hypothesis that 5-LOX-activated pro-inflammatory cytokine cascades promote nigrostriatal damage in the mouse MPTP model, the precise AA metabolites that contribute to degeneration remain unknown and a role for oxidative damage cannot be ruled out. In fact, DA turnover, which promotes the formation of reactive and cytotoxic DA quinones (Graham, 1978; Ogawa et al., 2000; Sulzer et al., 2000), is lowered in null 5-LOX vs. WT mice exposed to MPTP (Fig. 2D) suggesting that oxidative stress may be a factor in 5-LOX-related vulnerability to exogenous insult.

Deficiency in 5-LOX function appears to be protective from MPTP challenge in the mouse line; however, evaluation under control conditions revealed provocative results: striatal DA, HVA and TH protein levels are significantly lower in null 5-LOX vs. WT littermates (Fig. 1A, C). These data demonstrate a relationship between 5-LOX activity and regulation of DA synthesis and metabolism, suggesting that 5-LOX activity contributes to normal striatal DA homeostasis. A previous *ex vivo* study revealed that AA-derived eicosanoids increase DA release in striatal slice cultures (Davidson, 2003); our finding is the first to show that 5-LOX activity is necessary for normal striatal levels of DA as well as its metabolite HVA and its synthetic enzyme TH.

Other studies indirectly provide evidence for a link between LOX activity and DA synthesis. For example, increases in 12-HETE inhibits protein kinase II (Piomelli et al., 1989), an enzyme which phosphorylates and activates TH (Fujisawa and Okuno, 1989). Whether 5-HETE has a similar effect on TH activity is unknown, but given the change in TH expression in null 5-LOX mice, it is likely additional mechanisms are at play. Reduced DA and TH expression were selective with 5-LOX isozyme deficiency; no change in either marker was revealed in mice lacking 12/15-LOX, although a subtle but consistent decrease in DOPAC was measured (Fig. 2B). These data support isozyme-specific functional activities, and importantly indicate that the 5-LOX isozyme elicits a beneficial or detrimental influence on the nigrostriatal dopaminergic pathway depending on cellular state (i.e. normal or distressed).

The seemingly paradoxical effects of 5-LOX activity can be reconciled, however. Previous studies *in vitro* revealed LOX catalyzes peroxidation of catecholamines to form toxic reactive species, but this deleterious activity was dependent on the presence of an oxidative insult (i.e. hydrogen peroxide) (Rosei et al., 1994). Extrapolating this finding, one interpretation is that the mitochondrial dysfunction caused by MPTP exposure may drive toxic LOX-mediated catecholamine modification or other deleterious effects and subsequent nigrostriatal damage.

Alternatively, LOX-mediated AA catabolism generates a variety of lipid products; these congeners have diverse function and can elicit pro- or anti-inflammatory cellular responses depending on their downstream targets. Increased expression and activity of 12-LOX accelerates pro-inflammatory gene expression including Cox2 (Prasad et al., 2008). 12-HETE activates signaling pathways related to localized oxidative stress, including glutathione depletion (Badr, 1997; Funk and Cyrus, 2001; Kuhn and O'Donnell, 2006), and antagonizes the antiinflammatory actions of PPARγ. Pro-inflammatory leukotrienes are also generated by 5-LOX; however, other downstream products of 5- and 15-LOX, lipoxins, act as agonists for PPARγ (Planaguma et al., 2002; Kuhn and O'Donnell, 2006). PPARγ agonists mediate protection through activating ROS-detoxifying enzymes and diminishing toxicant-induced increased expression of cytokines in animal models of disease, including MPTP (St-Pierre et al., 2006; Schintu et al., 2009; Sobrado et al., 2009; Carta et al., 2011). These results suggest that future studies should regard the 5-LOX isozyme and its products as potential targets in the study of nigrostriatal vulnerability.

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Abbreviations

LOX	lipoxygenase(s)
AA	arachidonic acid
PD	Parkinson's disease
AD	Alzheimer's disease
WT	wildtype
TH	tyrosine hydroxylase
MPTP	1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine
GFAP	glial acidic fibrillary protein
ROS	reactive oxygen species
HPETE	hydroxyeicosatetraenoic acid
HETE	hydroxyeicosatetraenoic acid
PPARγ	peroxisome proliferator activator receptor gamma
LTB_4	leukotriene B4
DA	dopamine
DOPAC	dihydroxyphenylacetic acid
HVA	homovanillic acid
ANOVA	analysis of variance

Highlights

- Deficiency in 5-, but not 12/15-, lipoxygenase is associated with reduced striatal dopamine and tyrosine hydroxylase levels
- 5-lipoxygenase deficiency protects against nigrostriatal damage from MPTP
- Diminished inflammatory response is noted in null 5-lipoxygenase vs. wildtype mice after intoxication by MPTP

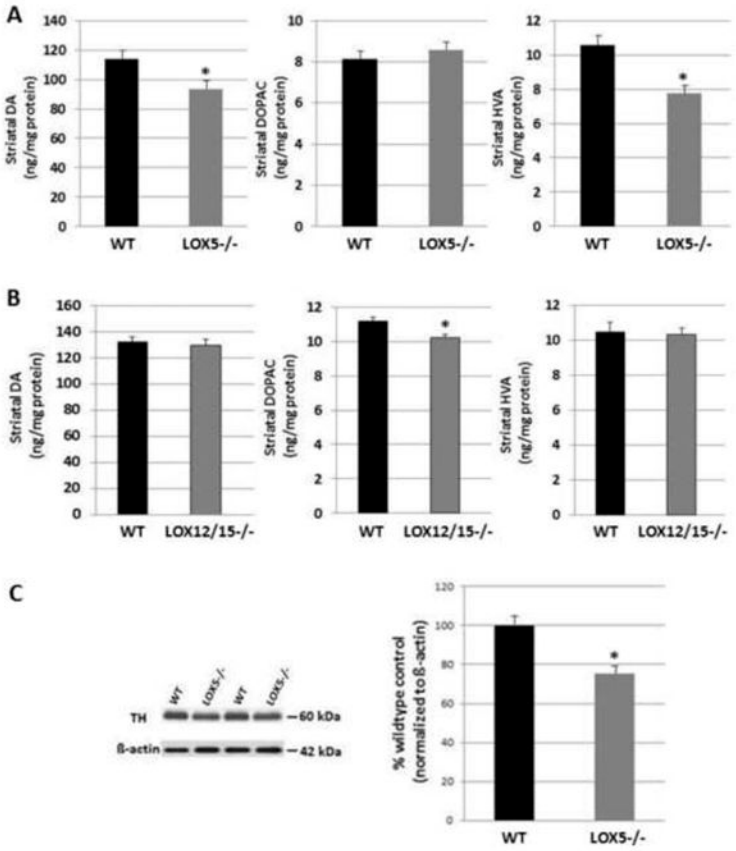

Fig. 1. LOX isozyme-selective effects on striatal dopaminergic tone

(A) DA and metabolites DOPAC and HVA were measured in striatal homogenates by reverse phase HPLC from WT and null 5-LOX littermates (n=6-8/group). (B) DA and metabolites DOPAC and HVA were measured in striatal homogenates from WT and null 12/15-LOX littermates (n=6-8/group). (C) The rate-limiting synthetic enzyme for DA, TH, was semi-quantitatively assessed using Western blot analyses of striatal homogenate from WT and null 5-LOX littermates (n=6-8/group). Immunoreactivity for TH was measured by optical density and normalized to the housekeeper β-actin. Data are shown as means±SEM. * $P<0.01$.

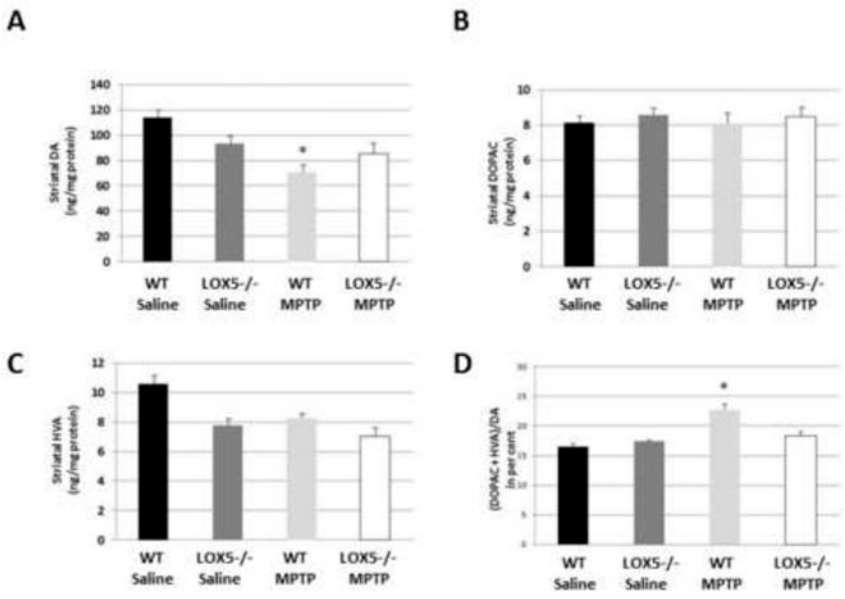

Fig. 2. 5-LOX isozyme effects on striatal neurochemistry following toxic insult

(A) DA was measured by reverse phase HPLC in WT and null 5-LOX littermates administered saline or MPTP (n=6-8/group). (B) DOPAC was measured by reverse phase HPLC in WT and null 5-LOX littermates administered saline or MPTP (n=6-8/group). (C) HVA was measured by reverse phase HPLC in WT and null 5-LOX littermates administered saline or MPTP (n=6-8/group). (D) DA turnover was assessed in WT and null 5-LOX littermates administered saline or MPTP (n=6-8/group). Data are shown as means±SEM. * $P<0.05$.

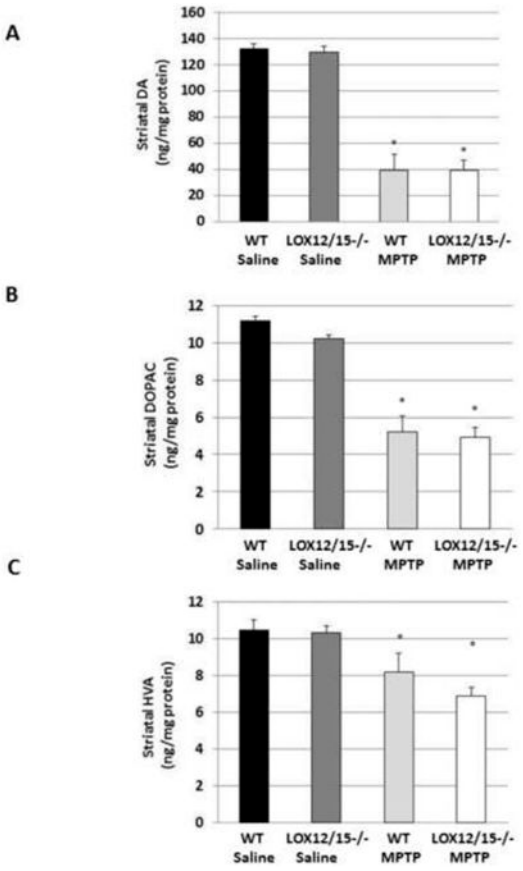

Fig. 3. 12/15-LOX isozyme effects on striatal neurochemistry following toxic insult

(A) DA, (B) DOPAC and (C) HVA were measured by reverse phase HPLC in WT and null 12/15-LOX littermates administered saline or MPTP (n=6-8/group). Data are shown as means±SEM. * $P<0.01$.

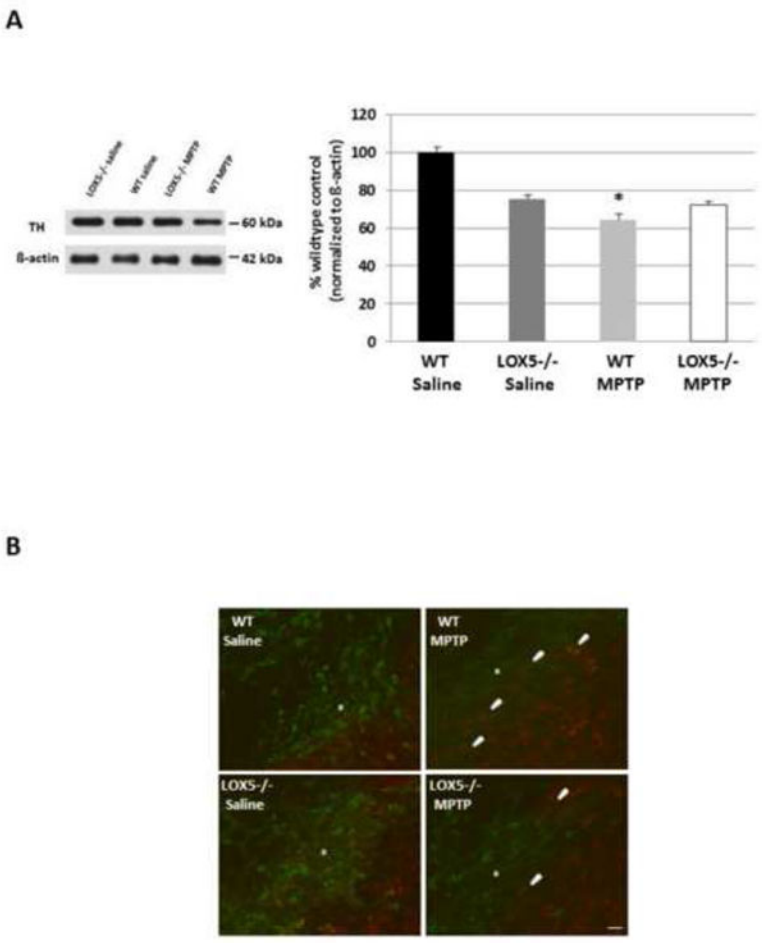

Fig. 4. 5-LOX isozyme effects on TH following toxic insult

(A) The rate-limiting synthetic enzyme for DA, TH, was semi-quantitatively assessed using Western blot analyses of striatal homogenate from WT and null 5-LOX littermates treated with saline or MPTP (n=6-8/group). Immunoreactivity for TH was measured by optical density and normalized to the housekeeper β-actin. Data are shown as means±SEM. * $P<0.05$. (B) Dual label immunofluorescence staining for TH (FITC) or GFAP (AF568) was performed in coronal brain sections containing substantia nigra from WT and null 5-LOX littermates treated with saline or MPTP. *indicates substantia nigra pars compacta; arrowheads show GFAP-immunoreactive astrocytes within the substantia nigra pars compacta. Bar = 25 μm.

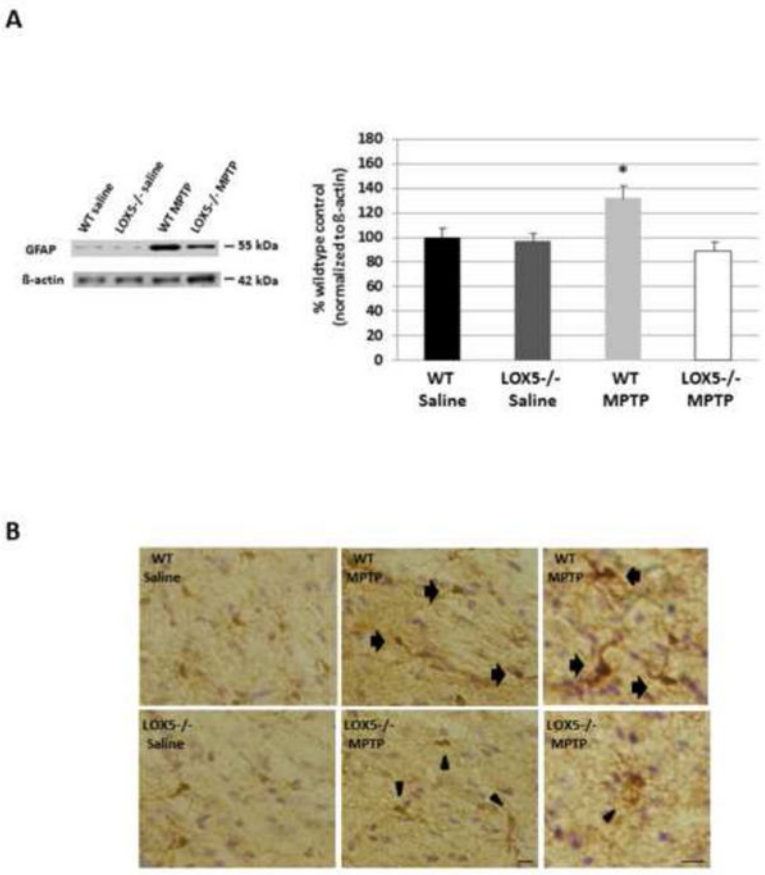

Fig. 5. 5-LOX isozyme effects on inflammatory markers following toxic insult

(A) The astrocytic protein GFAP was semi-quantitatively assessed using Western blot analyses of striatal homogenate from WT and null 5-LOX littermates treated with saline or MPTP (n=6-8/group). Immunoreactivity for GFAP was measured by optical density and normalized to the housekeeper β-actin. Data are shown as means±SEM. * $P<0.05$. (B) Immunohistochemical staining for the microglial protein Iba-1 was visualized using the brown chromogen DAB with counterstaining by cresyl violet. Coronal brain sections containing substantia nigra from WT and null 5-LOX littermates treated with saline or MPTP were utilized. Panel on far right shows microglial morphology: arrows indicate rounded cell bodies with short, thick processes; arrowheads denote ramified cell bodies with long, branching processes. Bar = 25 μm.