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Examining the role of paraoxonase 2 in the dopaminergic system of the mouse brain

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Abstract

Background: Paraoxonase 2 (PON2) is an intracellular antioxidant enzyme located at the inner mitochondrial membrane. Previous studies have found PON2 to be an important antioxidant in a variety of cellular systems, such as the cardiovascular and renal system. Recent work has also suggested that PON2 plays an important role in the central nervous system (CNS), as decreased PON2 expression in the CNS leads to higher oxidative stress and subsequent cell toxicity. However, the precise role of PON2 in the CNS is still largely unknown, and what role it may play in specific regions of the brain remains unexamined. Dopamine metabolism generates considerable oxidative stress and antioxidant function is critical to the survival of dopaminergic neurons, providing a potential mechanism for PON2 in the dopaminergic system.

Methods: In this study, we investigated the role of PON2 in the dopaminergic system of the mouse brain by comparing transcript and protein expression of dopaminergic-related genes in wildtype (WT) and PON2 deficient (PON2-def) mouse striatum, and exposing WT cultured primary neurons to dopamine receptor agonists.

Results: We found alterations in multiple key dopaminergic genes at the transcript level, however many of these changes were not observed at the protein level. In cultured neurons, PON2 mRNA and protein were increased upon exposure to quinpirole, a dopamine receptor 2/3 (DRD2/3) agonist, but not fenoldopam, a dopamine receptor 1/5 (DRD1/5) agonist, suggesting a receptor-specific role in dopamine signaling.

Conclusions: Our findings suggest PON2 deficiency significantly impacts the dopaminergic system at the transcript level and may play a role in mitigating oxidative stress in this system further downstream through dopamine receptor signaling.

Keywords: Paraoxonase 2 (PON2), Transcript, Protein, Dopamine, Agonist, Antagonist

Background

Paraoxonase 2 (PON2) is a member of the paraoxonase family consisting of three genes: PON1, 2 and 3. All three genes align in tandem on mouse chromosome 6 and the long arm of human chromosome 7q21-22 [1]. Although the family was named for the esterase activity of PON1 against paraoxon, the active metabolite of

the organophosphate insecticide parathion, PON2 possesses minimal esterase activity and does not hydrolyze paraoxon. However, all three PONs possess lactonase activity, exhibiting overlapping but distinct substrates for lactone hydrolysis [2]. While PON1 is a circulating enzyme associated with high-density lipoproteins (HDL) in plasma and PON3 both circulates and expresses intracellularly, PON2 does not circulate and is an intracellular, membrane-bound enzyme located at the inner mitochondrial membrane [3–5]. Here, PON2 associates with co-enzyme Q₁₀ and is thought to play a role maintaining redox balance during oxidative phosphorylation [5]. In neurons and astrocytes, PON2 has also been found at the

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cell membrane [6], but the function of PON2 at this location, and whether it differs from that of the mitochondrial membrane, is unknown. It is ubiquitously expressed and observed in all tissues examined, with the highest expression found in the lung, liver, intestines, heart, brain and kidneys respectively [6]. PON2 expression has been reported as sexually dimorphic, with females displaying significantly higher levels of PON2 than males [7, 8]. However, this expression pattern has not been consistent, with some studies reporting no difference [9].

Previous studies have demonstrated PON2 is a critical antioxidant enzyme, as the loss of PON2 is associated with multiple morbidities in-vivo, such as atherosclerosis [5, 10], heart failure [11], impaired insulin signaling [12, 13], and obesity [14]. Epidemiological studies have also linked PON2 SNPs with Alzheimer's disease [15], suggesting an important role in the brain. Although the mechanism by which PON2 acts as an antioxidant has yet to be fully understood, prevailing evidence supports PON2 acts by inhibiting semiquinone-mediated superoxide formation [16]. Inhibition of radical formation is of particular importance in the dopaminergic system of the central nervous system (CNS) where dopamine (DA) metabolism generates free radicals and reactive quinones through DA oxidation [17]. This continuous oxidative burden from DA metabolism renders dopaminergic neurons more vulnerable to additional oxidative stressors and cellular death [18]. Indeed, a leading hypothesis on the etiology of Parkinson's disease (PD) suggests uncontrolled oxidative stress cascades leading to dopaminergic neuron death likely play a critical role in disease pathology [19]. This hypothesis is supported by significant in-vivo evidence of higher oxidative stress markers in the brains of human PD patients [20] and genetic mouse models [21]. Neurotoxicant animal models mimic PD via exposure to 6-hydroxydopamine (6-OHDA), 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP), or agropesticides which cause mitochondrial dysfunction and/or oxidative stress to destroy dopaminergic neurons [19], highlighting the importance of both redox balance and mitochondrial function for survival and proper function of dopaminergic neurons.

PON2 has been shown to be differentially expressed in the brain, with mice having significantly higher PON2 in dopaminergic regions such as the striatum and substantia nigra [6]. In-vitro PON2 has been shown to interact with DJ-1 (PARK7) and the protective antioxidant effects of DJ-1 are mediated partly through PON2 expression [22]. DJ-1 is implicated in PD etiology and loss-of-function mutations of DJ-1 account for 1% of all familial PD cases; whether PON2 plays a role in PD etiology is currently unknown and no additional studies related to PON2 and PD exist in the literature. In the kidney,

studies have shown PON2 plays an important antioxidant role in the renal dopaminergic system, a critical system for maintaining homeostatic blood pressure [23]. Activation of dopamine receptors 2 and 5 (DRD2 and DRD5) upregulates PON2 expression which in turn inhibits renal NADPH oxidases, pro-oxidant enzymes that produce ROS [24, 25]. Maintenance of redox balance by antioxidants such as PON2 is important as unhampered ROS in the renal system can have detrimental effects to both electrolyte balance and systemic blood pressure.

Considering the expression pattern and antioxidant functionality of PON2, as well as evidence from other organ systems, it is feasible that PON2 may play an important role in the dopaminergic system of the CNS. Minimal studies have addressed the role of PON2 in the CNS, despite in-vivo and epidemiological evidence suggesting its importance. To address whether PON2 plays a role in this system, we examined wildtype (WT) and PON2 deficient (PON2-def) striatum from mice and compared the transcript and protein levels of key dopaminergic-related targets, to determine if PON2 deficiency leads to expression changes, specifically within the nigrostriatal dopaminergic system. The striatum was chosen as the tissue of interest as it is the primary active site of dopaminergic signaling for the nigrostriatal pathway, where dopamine synthesis and release occurs in the synaptic terminals of innervating neurons from the substantia nigra pars compacta (SNc), and the receiving dopamine receptors lie on the striatal interneurons. Additionally, we conducted in-vitro experiments with cultured primary neurons to determine if PON2 expression was modulated upon exposure to two selective dopamine receptor (DR) agonists, fenoldopam (DRD1/5) and quinpirole (DRD2/3).

Materials and methods

Materials

Anti-DRD1, DRD2, DRD5, DAT, HO-1, HO-2, NOX2, TH and VMAT2 antibodies were purchased from Abcam (Cambridge, MA, USA). Anti-PON2 antibody was purchased from Genscript (Piscataway, NJ, USA). Anti β -actin antibody, papain, poly-D-lysine, quinpirole hydrochloride, fenoldopam mesylate and L-741,626 were purchased from MilliporeSigma (Burlington, MA, USA). Anti-rabbit IgG HRP-linked antibody and Cell Lysis Buffer 10 \times were purchased from HRP Goat Anti-Mouse IgG was purchased from BD Biosciences (San Jose, CA, USA). XCell II Blot Module, XCell SureLock Electrophoresis Cell, NuPAGE MOPS SDS Running Buffer 20x, NuPAGE LDS Sample Buffer 4x, NuPAGE Antioxidant, NuPAGE Sample Reducing Agent 10x, NuPAGE 10% Bis-Tris Protein Gels, Neurobasal-A medium, B-27 supplement with and without antioxidant, fungizone, HBSS

medium (No Ca^{2+}) and 10 mM HEPES were purchased from Life Technologies (Carlsbad, CA, USA). Immobilon-P Transfer Membrane was purchased from Millipore Corporation (Billerica, MA, USA). Restore Western Blot Stripping Buffer, PageRuler Prestained Protein Ladder, SuperSignal West Pico Chemiluminescent Substrate and Costar 6-well TC plates were purchased from Thermo Fisher Scientific (Waltham, MA, USA). RNeasy Mini Kit was purchased from Qiagen (Hilden, Germany). iScript cDNA Synthesis Kit, iTaq Universal SYBR Green Supermix and CFX384 Real-Time Detection System were purchased from Bio-Rad Laboratories (Hercules, CA, USA). GraphPad Prism software version 9.1.2 was purchased from GraphPad Software Inc. (San Diego, CA, USA).

Animals, tissue and cell culture

Mice

Wild-type (WT) and PON2 deficient (PON2-def) mice [10] on a C57BL/6 J background were used for this study. $N=5$ mice per sex per genotype were utilized, with male and female mice combined for analysis if no sex difference was observed. Mice were euthanized at postnatal day (PND) 60 using carbon dioxide (CO_2) followed by decapitation. Striatal tissue was dissected and flash frozen in liquid nitrogen. Tissue was pulverized with a pre-chilled mortar and pestle into a fine powder, stored at -80°C and aliquoted into appropriate lysis buffers for protein and RNA extraction as needed, utilizing sonication for homogenization when indicated. Mice were housed in a specific pathogen-free facility on a 14-h light/10-h dark cycle with ad libitum access to food and water. All procedures were conducted in accordance with the National Institute of Health Guide for the Use and Care of Laboratory Animals and were approved by the University of Washington Institutional Animal Care and Use Committee (IACUC) under protocol #2077-14. Animal numbers were kept to a minimum and all efforts were made to reduce animal suffering.

Primary cell culture

Cerebellar granule neurons (CGNs) were prepared from PND 7 mice, as previously described [26]. A total of 3–5 pups were pooled per cell preparation, with male and female pups prepared separately. In this study, 4 cell preparations (2 female, 2 male) were used. Neurons were grown for 10–12 days before treatments. Briefly, cerebellar tissue was collected in chilled HBSS medium (No Ca^{2+}) and 10 mM HEPES. Tissues were digested for 30 min in HBSS containing papain (1 mg/mL) and DNase I (40 $\mu\text{g}/\text{mL}$) and centrifuged at $300\times g$ for 7 min at room temperature. The supernatant (containing papain) was removed, and the pellet was gently triturated

in serum-free Neurobasal-A Medium supplemented with B27 (Life Technologies, Carlsbad, CA). Cells were centrifuged at $200\times g$ at 4°C for 10 min and the cell pellet was gently resuspended in medium. Neurons were then counted, seeded on poly-D-lysine coated 6-well plates at a density of $5\times 10^4/\text{cm}^2$, and cultured in serum-free Neurobasal-A medium supplemented with B27. After 4 days, media was completely exchanged for serum-free Neurobasal-A Medium with B27 minus antioxidants ($-AO$). Concentrations for the agonist/antagonist experiments were determined from a set of preliminary experiments (data not shown), with a starting concentration of $1\ \mu\text{M}$ chosen from existing literature [24, 25]. $1\ \mu\text{M}$ quinpirole was not sufficient to increase PON2 expression in primary neurons, while $3\ \mu\text{M}$ and $20\ \mu\text{M}$ quinpirole were. 1 , 3 and $20\ \mu\text{M}$ fenoldopam did not affect PON2 expression. $3\ \mu\text{M}$ quinpirole was chosen for the remaining experiments as it was the lowest concentration which modulated PON2 expression in our preliminary examination, and no difference in expression was found between $3\ \mu\text{M}$ and $20\ \mu\text{M}$ quinpirole. Quinpirole in low doses ($\leq 100\ \text{nM}$) is demonstrated to selectively target autoreceptors in-vivo when injected into mice [27, 28]. All of our investigated concentrations are considered high by existing literature standards and are anticipated to reduce autoreceptor binding and preferentially target postsynaptic receptors. For the DRD1 and DRD2 agonist experiments, neurons were treated for 24 h with fenoldopam mesylate ($3\ \mu\text{M}$) or quinpirole hydrochloride ($3\ \mu\text{M}$). For the DRD2 antagonist experiment, neurons were pre-treated for 1 h with L-741,626 ($3\ \mu\text{M}$) prior to a 24-h co-incubation with L-741,626 ($3\ \mu\text{M}$) and quinpirole ($3\ \mu\text{M}$) together.

Immunoblotting

Immunoblots were carried out as previously described [29]. Briefly, 5 mg of pulverized striatum was homogenized in $1\times$ Cell Lysis Buffer (Cell Signaling Technology, Danvers, MA) and 15 μg of protein was mixed with SDS running buffer and sample reducing agent and subjected to sodium dodecyl sulfate–polyacrylamide gel electrophoresis (SDS-PAGE). Following electrophoresis, proteins were transferred to polyvinylidene difluoride membranes, and the membrane blocked for 1–3 h with 5% nonfat milk. Membranes were then probed with the following diluted primary antibodies: PON2 1:2000, DRD1 1:10,000, DRD2 1:2500, DRD5 1:2500, TH 1:3000, VMAT2 1:1500, DAT 1:1000, HO-1 1:5000, HO-2 1:3000 and NOX2 1:12,000. Following primary antibody incubation, membranes were washed with Tris-buffered saline with 0.1% Tween-20 ($\text{pH}=7.5$) and incubated with horseradish peroxidase-conjugated anti-rabbit secondary antibody at the following dilutions: PON2 1:5000, DRD1

1:5000, DRD2 1:5000, DRD5 1:5000, TH 1:3000, VMAT2 1:3000, DAT 1:5000, HO-1 1:4000, HO-2 1:3000 and NOX2 1:4000. Membranes were stripped with Restore™ Western Blot Stripping Buffer (Thermo Fisher Scientific, Waltham, MA) and re-probed for β -actin using a dilution of 1:2500 for the β -actin primary antibody and 1:2500 for the horseradish peroxidase-conjugated anti-mouse secondary antibody. Intensity of bands was measured by densitometry using ImageJ software (NIH), with the band intensity normalized to β -actin expression.

RT-PCR

Total RNA was extracted from striatal tissue using the RNeasy Mini Kit (Qiagen, Hilden, Germany) according to the manufacturer's established protocol. Target mRNA levels were measured by RT-PCR, see Table 1 for sequences of primer pairs. cDNA was generated from 1 μ g total RNA using the iScript cDNA Synthesis Kit (Bio-Rad Laboratories, Hercules, CA) according to the manufacturer's established protocol. The cDNA samples were diluted to a concentration of 6.25 ng/ μ L with nuclease-free water and subsequently used for quantitative polymerase chain reaction (qPCR) using iTaq Universal SYBR Green Supermix in a CFX384 Real-Time Detection System (Bio-Rad Laboratories, Hercules, CA). 6 μ L of diluted cDNA were included in a PCR reaction mastermix containing 15 μ L Universal SYBR Green Supermix (2x), 1.5 μ L each forward and reverse primers (10 μ M stock), and 6 μ L nuclease-free water (30 μ L final volume). The reaction mixture was then aliquoted in triplicate, 8 μ L per reaction per sample. The thermal cycling conditions were as follows: A single denaturing step at 95 °C for 30 s, 40 cycles of 95 °C for 15 s, 60 °C for 30 s and 72 °C for 30 s. dCq

values (referenced as “relative mRNA”) for each target were calculated by subtracting Cq values of housekeeping gene GAPDH. The stability of GAPDH was assessed using the NormFinder algorithm in Excel [30] and found to be stably expressed between WT and PON2-def tissue, in both males and females (Additional file 1).

Statistical analysis

Statistical analyses were conducted using GraphPad Prism software (version 9.1.2, GraphPad Software Inc., San Diego, CA, USA). Data are expressed as the mean \pm SEM and all graphed results represent biological replicates. For immunoblotting and qPCR experiments, tissue from at least 5 animals per group was included. For primary cell culture experiments, 4 independent experiments were analyzed, with each experimental cell population derived from a different animal cohort. One-way ANOVA followed by the Bonferroni correction for multiple comparisons was utilized for statistical analysis of multiple groups, while Student's t-test was utilized for comparing two groups as noted. Prior to ANOVA analysis, data normality was assessed using the Shapiro–Wilk test.

Results

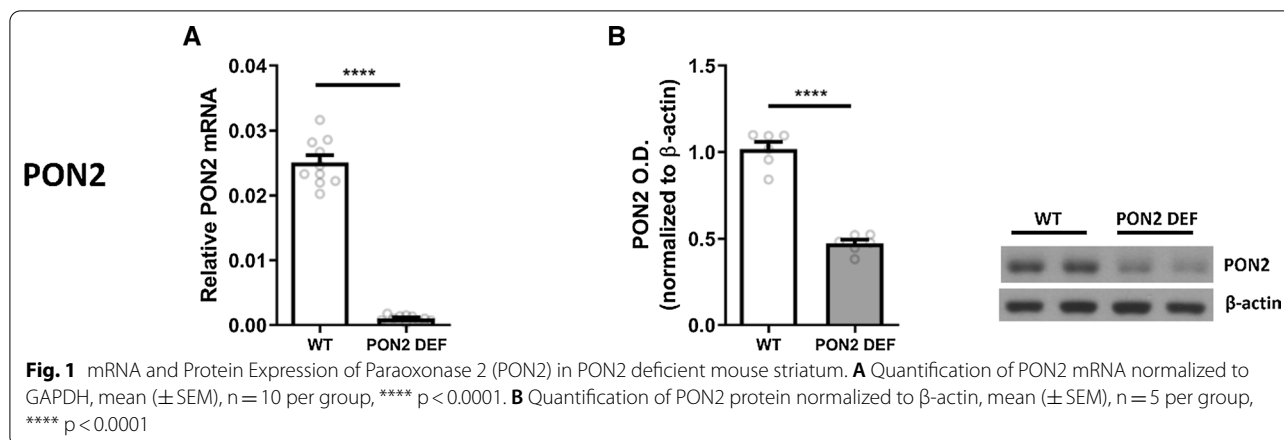
Verification of PON2 deficiency

PON2 expression

To verify if the mice used for this study were PON2 deficient, we measured PON2 transcript and protein expression in WT and PON2-def mice. The PON2-def mice had nearly 30 times lower transcript expression of PON2 (Fig. 1A), and 2–3 times lower protein expression (Fig. 1B).

Table 1 RT-PCR primer pair sequences

Target	Forward Primer (5'–3')	Reverse Primer (5'–3')
PON2	GCACGCTGGTGACAAATTTATC	TGTCAGTATGGCTTCTCGGAT
DRD1	TCTTTGTCATCTCTTTAGCTGTGTC	TTCGGAGTCATCTTCTCTCA
DRD2	ATCTCTTGCCCACTGCTCTTTGGA	ATAGACCAGCAGGGTGACGATGAA
DRD5	ACCAAGACACGGTCTTCCAC	CCTCCTCTCACAGTCAAGC
TH	CAGCTGGAGGATGTGTCTCA	GGCATGACGGATGTACTGTG
VMAT2	TCATCGCTGCAGGCTCCATCT	AGTGCCCACTTTCGGGAACAC
DAT	GGGTGGCCTGGTTCTACG	CAGCATAGCCGCCAGTACAG
HO-1	GCCGAGAATGCTGAGTTTCATG	TGGTACAAGGAAGCCATCACC
HO-2	TACTTCACATACTCAGCCCT	ATGGGCCACCAGCAGCTCTG
NOX2	CAGGAACCTCACTTTCCATAAGATG	AACGTTGAAGAGATGTGCAATTGT
GAPDH	TGACCTCAACTACATGGTCTACA	CTTCCATTCTCGGCCTTG



Comparing dopaminergic targets in PON2 deficient and wildtype mice

Dopamine receptors

In the striatum, PON2-def mice had significantly higher levels of DRD1 (Fig. 2A) and DRD5 (Fig. 2E) transcript compared to WT mice, while DRD2 (Fig. 2C) levels were identical. To further investigate changes at the protein level, these targets were also measured using Western blot analysis. However, no differences were seen when comparing protein levels of any dopamine receptor D1, D2 and D5 respectively (Fig. 2B, D, F).

Dopamine metabolism and transport

Various genes involved in DA metabolism and transport were measured at the transcript and protein level using RT-PCR and Western blot analysis. Transcript for tyrosine hydroxylase (TH), the rate limiting enzyme in DA metabolism, was measured and found to be significantly higher in PON2-def striatum compared to WT (Fig. 3A), with an opposite effect observed in the measured protein levels, where PON2-def striatum had decreased TH protein compared to WT (Fig. 3B). Vesicular monoamine transporter 2 (VMAT2), the principle solute carrier which shuttles DA from the cytosol into synaptic vesicles for release at the presynaptic membrane, was found to have significantly higher transcript levels in PON2-def striatum compared to WT (Fig. 3C), although there were no significant differences in the protein levels (Fig. 3D). Dopamine transporter (DAT), the synaptic transmembrane transporter which recovers DA from the synaptic cleft back into the cytosol, was unchanged at both the transcript (Fig. 3E) and protein level (Fig. 3F).

Antioxidant enzymes

Oxidative balance is of particular importance in dopaminergic neurons, and we sought to determine if PON2

deficiency affects the expression of specific antioxidant enzymes in the striatum. Transcript levels of heme-oxygenase 1 (HO-1) and 2 (HO-2) were significantly higher in PON2-def striatum compared to WT (Fig. 4A, C), while no differences were observed at the protein level (Fig. 4B, D). Similarly, NADPH oxidase 2 (NOX2) transcript levels were significantly increased in PON2-def mice (Fig. 4E), while the protein levels of NOX2 were unchanged (Fig. 4F).

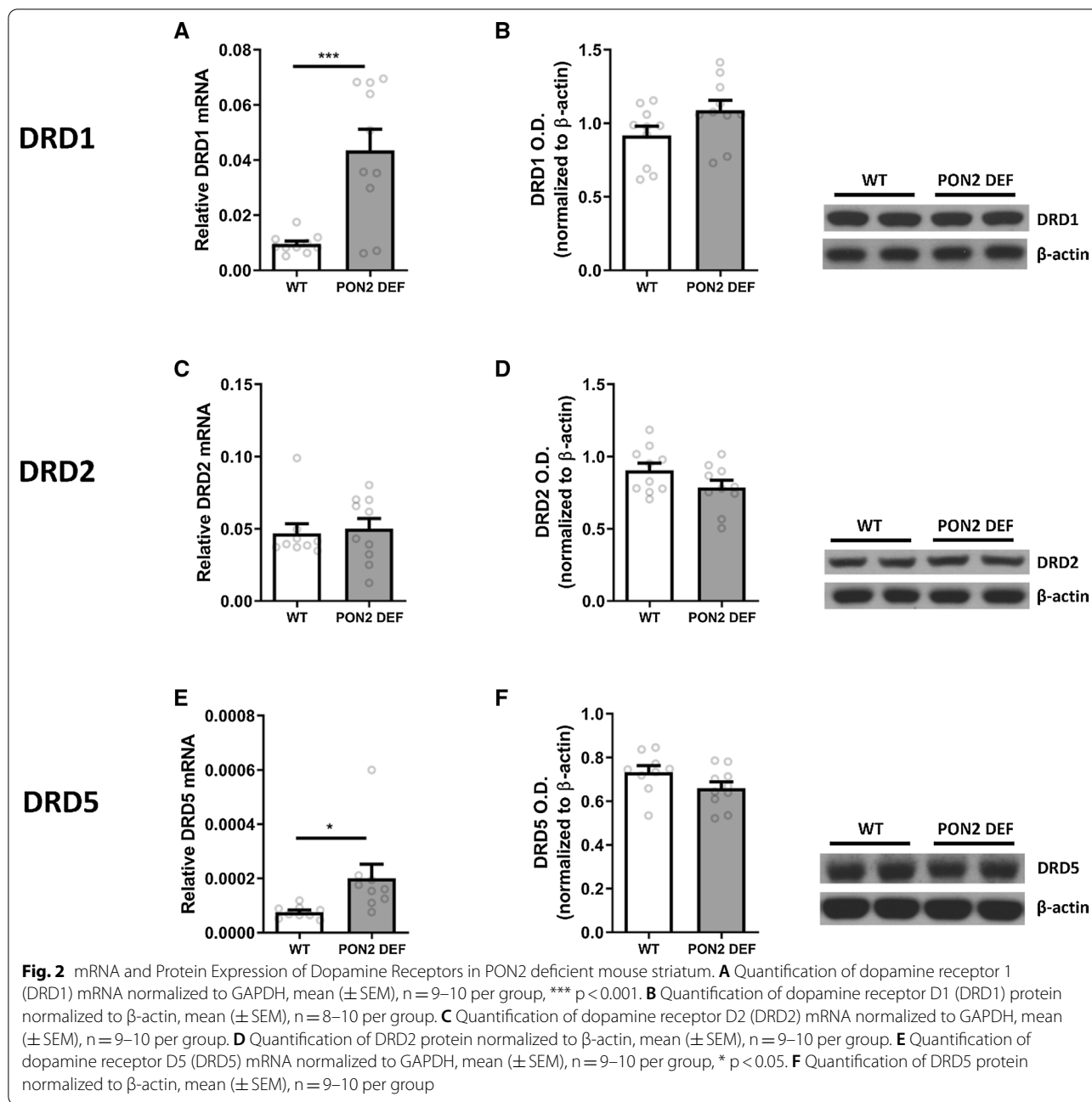
PON2 expression in CGNs after dopamine receptor agonist exposure

Dopamine receptor D1 and D5 signaling

To investigate whether PON2 may be modulated by DA signaling, we exposed neurons to a DRD1/5 agonist, fenoldopam, and measured PON2 transcript and protein. A 24-h incubation with 3 μ M fenoldopam did not alter PON2 expression compared to untreated cells at the transcript (Fig. 5A) or protein level (Fig. 5B). Higher concentrations of up to 20 μ M also did not affect PON2 expression (data not shown).

Dopamine receptor D2 signaling

We also evaluated whether PON2 could be modulated by DRD2 signaling by exposing primary neurons to the DRD2/3 agonist quinpirole. A 24-h incubation with 3 μ M quinpirole significantly increased the expression of PON2 at both the transcript and protein level (Fig. 6A and B). A 1-h pre-treatment and 24 h co-exposure with a competitive DRD2 antagonist, L-741,626, was able to abolish the increased PON2 expression (Fig. 6A, B). These findings support that the observed expression increase of PON2 was due to activation of DRD2, and not that of DRD3 or non-selective binding to other targets.

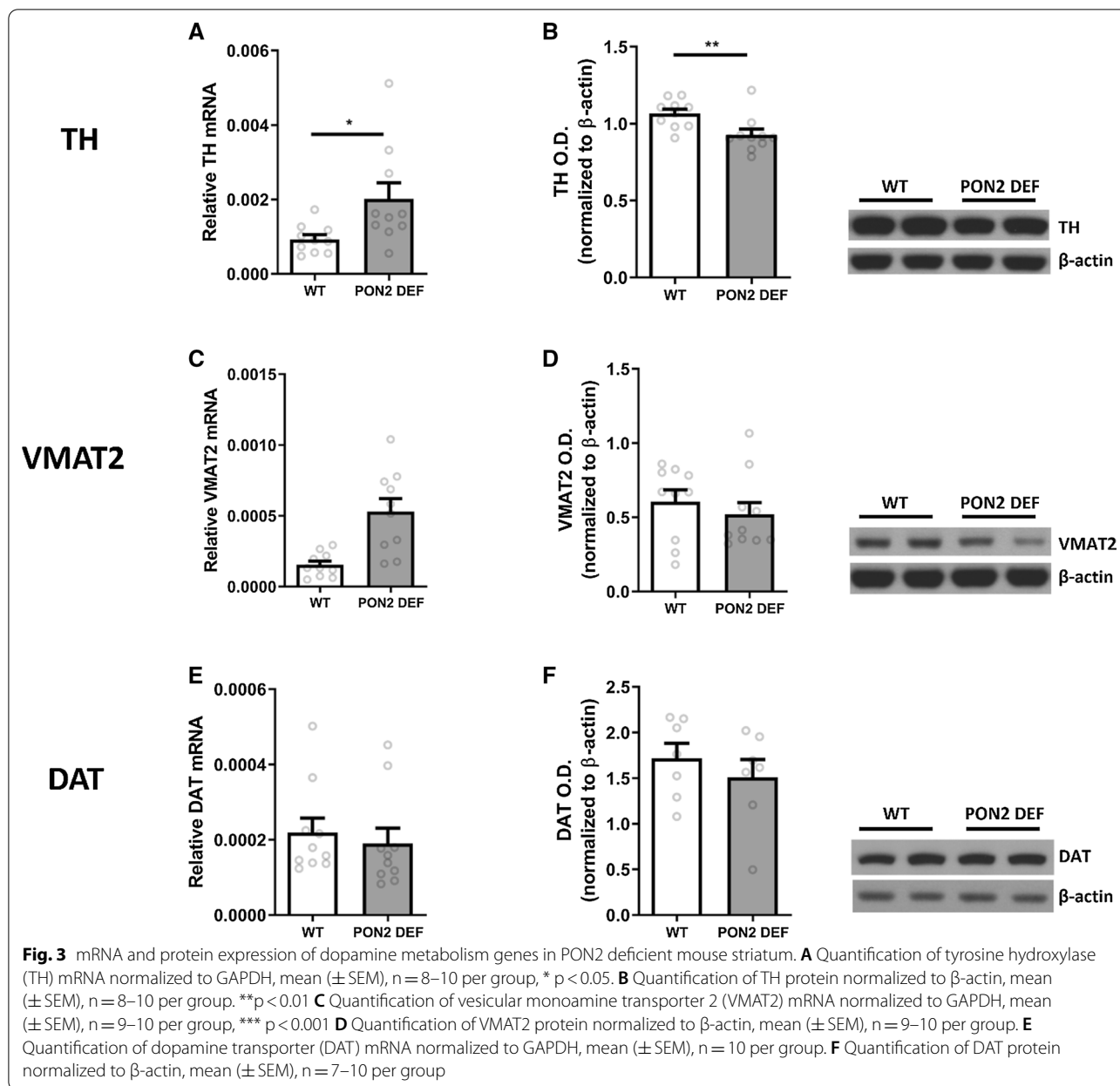


Discussion

While widely expressed in the brain, PON2 levels are the highest in dopaminergic regions such as the striatum, suggesting a function in these areas [6]. Feasibly, PON2 expression may be higher in these regions due to the increased oxidative stress burden stemming from DA metabolism, a process which generates ROS [17]. Using a PON2 deficient mouse model, we have shown that PON2 deficiency in the striatum significantly impacts multiple dopaminergic-related genes at the transcript level,

upregulating DRD1, DRD5, TH and VMAT2. Transcript levels of antioxidant enzymes NOX2, HO-1 and HO-2 were also significantly increased in PON2-def mice, suggesting these animals may have higher burdens of oxidative stress in the striatum.

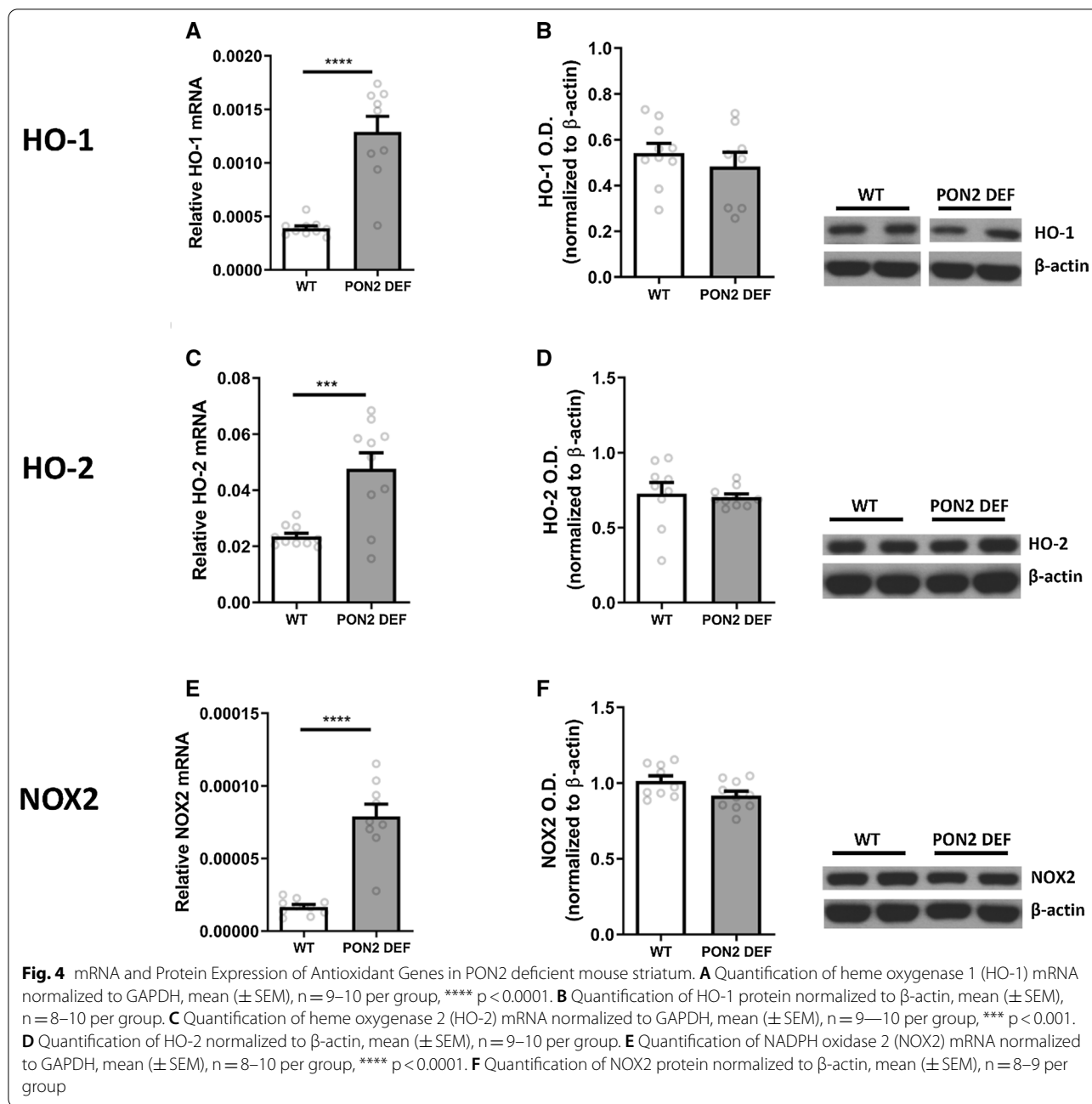
Although expressing significantly less PON2 transcript and protein compared to WT mice, the PON2-def mouse is not a full knockout model, with these animals producing a small amount of PON2. It is currently unknown what amount of PON2 loss is necessary



before dysfunction occurs in a given system, and it is possible that this deficient model does not reach that threshold for overt impacts to the dopaminergic system. Moreover, it is unknown how stable this deficiency is over time. PON2-def mice may experience periods of oscillating deficiency over their lifetime, and our single-time point analysis in this study would be unable to capture such dynamic changes. To eliminate many of these issues, utilization of a full knockout model would be ideal for investigation of PON2 going forward, with incorporation of the PON2-def model for later analysis. Indeed, the deficient model does have translational investigation

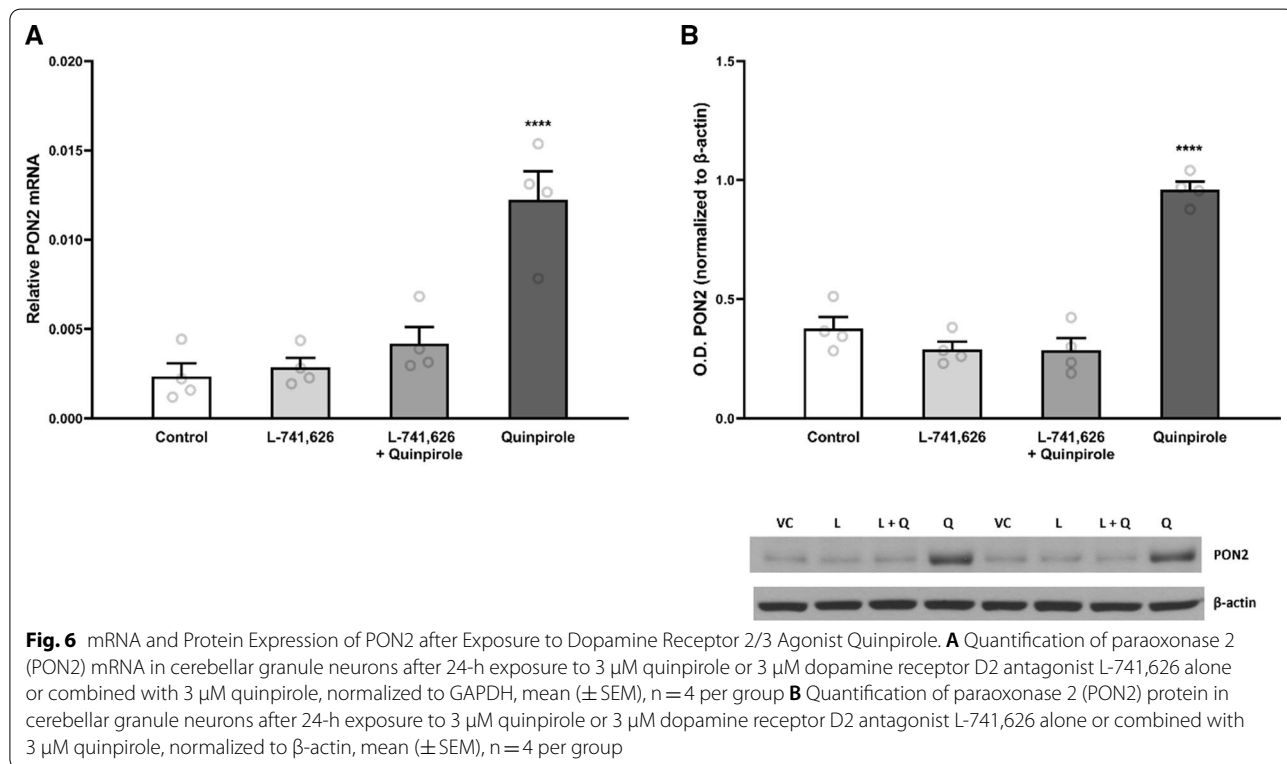
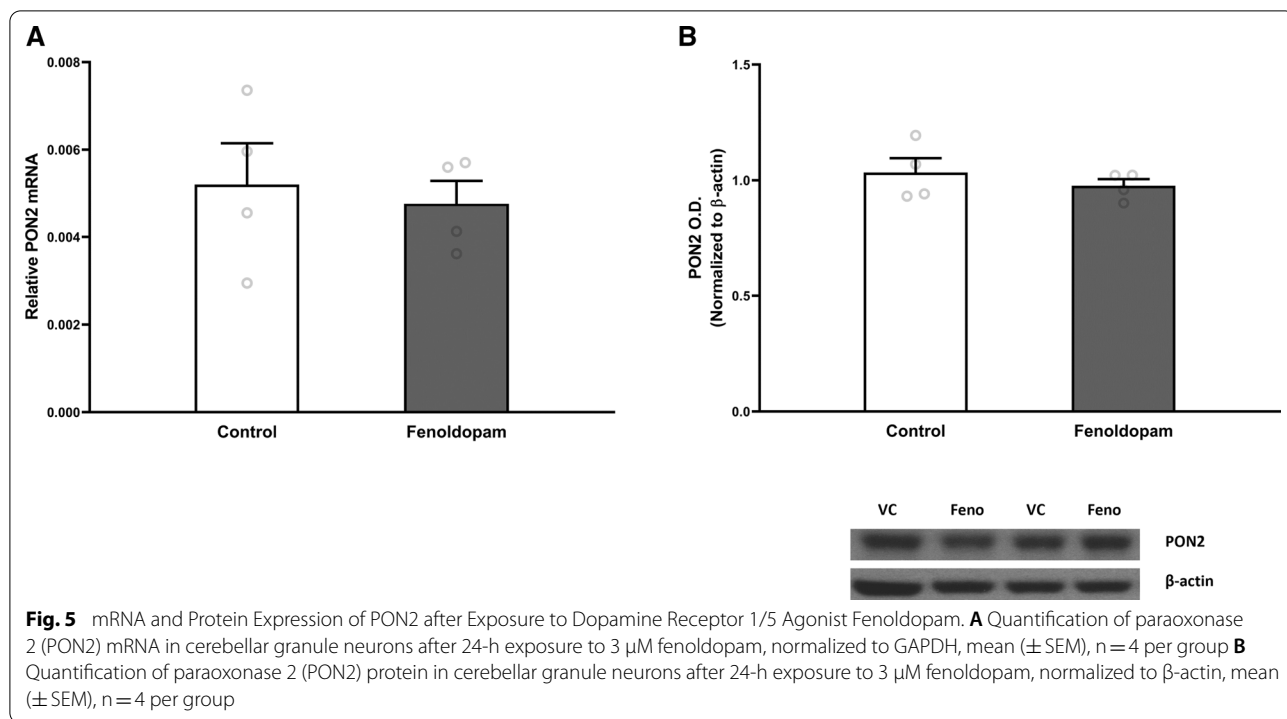
benefits, as PON2 polymorphisms identified in the general population are believed to reduce the enzymatic activity of PON2, but do not abolish its expression [31]. In the absence of a humanized knock-in model, the deficient model may be the best functional approximation of these polymorphisms to study the translational aspects of PON2.

Notably, no sex differences were observed in our study, and male and female data was subsequently combined for analysis. PON2 expression has been reported in the literature to be higher in females in both mice [7] and monkeys [8]. However, our present study did not identify



differential expression of PON2 between WT females and males, and sex was not a modulating factor when comparing dopaminergic gene expression between WT and PON2-def mice. These results are consistent with a recent study from our group comparing brain region RNA expression between WT and PON2-def mice [9], where a sex difference in PON2 expression was also not observed. Estrogen is thought to be the driving factor behind sex-based PON2 expression differences, as removing the ovaries of female mice leads to a reduction

in PON2 levels [7]. Potentially, the estrus cycle is an important regulator of PON2, and sex-based expression differences may be linked to the estrus cycle and fluctuations in circulating estrogen levels of female animals. Estrus cycle status of females was not accounted for in our study, but would be worthwhile to note for future studies. There is also evidence that PON2 expression may be under the control of circadian regulators [9], and the time of tissue collection could be an important factor for PON2 expression measurements.



Despite significant increases in transcript with PON2 deficiency, most targets were unchanged at the protein level in the striatum. In the case of TH, the transcript and

protein effects observed were opposite and PON2-def mice may have a reduction in DA metabolism because of lower TH protein expression, although analysis of DA

metabolites would be necessary to confirm this. In addition to metabolites, measurement of the DA catabolic pathway would be of interest for this tissue but was not fully addressed in our study. We have found no changes in monoamine oxidase B (MAOB) expression with PON2 deficiency (Additional file 2), but we have not investigated the expression of monoamine oxidase A (MAOA), the primary catabolizer of DA in rodents [32, 33]. Future studies probing the role of PON2 in this system would benefit from the inclusion of catabolic pathways to understand impacts to the full lifecycle of DA.

The reported discrepancies in transcript and protein could be the result of multiple factors and are not uncommon, as correlation between transcript and protein is known to be poor, with Pearson correlation coefficients reported as low as $R=0.36$ [34]. Recent work looking at global transcript and protein expression in the human brain also supports this, identifying a Pearson correlation coefficient ranging from $R=0.32-0.5$ [35]. An important factor for this poor correlation identified by Carlyle et al. is the vast neuronal projection network throughout the brain, where the cell bodies of neurons reside in one region and the synaptic terminals in another. This is of relevance for our study as well, as many of the targets we analyzed for DA metabolism are synaptic proteins. These synaptic terminals in the striatum originate from neurons in other regions of the brain, such as the substantia nigra pars compacta (SNc) in the case of dopaminergic input. Thus, our usage of striatal tissue includes the axons and synaptic terminals of SNc neurons but excludes their cell bodies. Our study did not look at the SNc, limiting our view of RNA expression within SNc neurons to transcripts which are locally translated at the synapse. Local transcript translation at the synapse is now recognized as an important function, particularly for neurons which project long distances and for which shuttling of proteins from the cell body down the axon may not be ideal for rapid signal transduction [36, 37]. TH has been experimentally shown to be locally translated [38], and other DA-related transcripts may follow suit for rapid signal transduction within this system. However, it is unknown when a transcript is preferentially translated locally, or when the protein is shuttled from the cell body. Since our study only analyzed striatal tissue, we are unable to comment on the expression changes that may be occurring in the SNc cell bodies of projected neurons. Expression data from the SNc would provide valuable insight into regional changes and identify potential discrepancies between translation occurring in the cell body versus the synaptic terminals. While RNA differences may be detected in striatal tissue, translation and shuttling of proteins may be occurring in the cell body from the SNc which negate any protein changes upon analysis

of striatal tissue. As well, striatal tissue includes striatal interneurons which express TH but are not dopaminergic [39], which may influence our expression results while not reflecting the dopaminergic system. Although the size of the interneuron population is low, around 10% of striatal cells [40], our methodology does not allow us to separate expression data from projection neurons versus interneurons. Sub-analysis of these populations would be of interest to determine if PON2 plays a differential role in projection neurons and interneurons.

An additional explanation for the observed transcript-protein discrepancy may be a temporal element to changes in the dopaminergic system that our study was unable to capture. Increased transcript may be present for quick translation and rapid increase in protein levels under physiological conditions that were not present at the time our tissue was collected. While transcript is expected to predict steady-state protein levels, defining 'steady-state' presents a challenge as cells are constantly adapting to their environment and can be in numerous states of change in a given period, such as during proliferation, differentiation or apoptosis [41]. Our study utilized whole striatal tissue comprised of multiple neural cell types, ranging from non-proliferating neurons to highly dynamic glial cells. Astrocytes are reported to have significantly higher expression of PON2 compared to neurons [6], and there may be underlying expression differences between these cell populations. Further investigation of specific cell types would be of interest to determine if there are differences in neural and glial populations, as well as additional time points after oxidative challenge to determine if these increases in transcript are primed for an adaptive response. Additionally, these discrepancies may point to a dysregulation in transcription mediation in PON2-def mice, where transcription is increased but translational controls maintain constant protein levels. This could be the result of increased activity of specific transcription factors to elicit transcription upregulation, while micro RNA (miRNA) activity and *trans*-acting proteins prevent increases of protein [42]. Our lab has recently identified that PON2 deficiency alters the expression of multiple targets related to transcription in the mouse brain, which could affect the expression of the targets analyzed in this study [9]. Excess protein may also be targeted for degradation through the ubiquitin-proteasome pathway to maintain proteostasis if previous translational controls are insufficient [43]. While increased transcript levels may not pose a risk when these controls are in place, loss of translational and chaperone control is noted in aged animals [44]. If these higher transcript levels persist for the lifespan of the PON2 deficient animal, effects on the dopaminergic system may be more apparent in old age as these control

systems break down and protein levels become aberrant. Increased expression of dopamine receptors may lead to signaling abnormalities and subsequent cognitive impairment [45] if translational controls break down and allow for the increased DRD1 and 5 transcripts to produce more protein. Further, oxidative damage over time from PON2 deficiency may significantly contribute to cellular dysfunction and exacerbate the effects of abnormal dopamine receptor expression. Oxidative stress is a hallmark of neurodegenerative disease, with both Parkinson's disease (PD) [19, 20] and Alzheimer's disease (AD) [46]. Additional investigation of the life stages of PON2-def mice, as well as proteostasis controls, would be of interest, with a particular focus on PON2 deficiency in aged animals and potential neurodegenerative consequences.

In our study, PON2 expression was significantly increased upon exposure to 3 μ M quinpirole, a DRD2/3 agonist, but not to 3 μ M fenoldopam, a DRD1/5 agonist. Dopamine receptors are G-protein-coupled receptors and can be divided into two families based on their pharmacologic properties and effects on cAMP production: D₁-like receptors (DRD1/5) and D₂-like receptors (DRD2/3/4). While D₁-like receptors couple to G stimulatory sites and increase cAMP production, D₂-like receptors couple to G inhibitory sites and inhibit both cAMP production and activity of adenylate cyclase [47]. The different dopamine receptors (DRD1–5) also display overlapping and distinct functions, with many playing a role in locomotion, attention, and cognition [48]. Effects on locomotion may be of particular relevance, as we have observed that PON2-def mice display locomotor deficits when tested on the rotarod [9]. However, locomotion is a complex behavior and involves multiple pathways, and PON2 deficiency in this pathway alone may not be sufficient to affect rotarod performance. Behavioral assessment of DRD2 knockout mice has shown that observed locomotor deficits were largely driven by strain differences, and not the absence of DRD2 itself [49], supporting that perturbation in multiple genes is necessary for overt phenotypic effects. Additional work to elucidate the function of PON2 in the DRD2 signaling pathway would be of value to determine if disruption to this pathway is responsible for the locomotor deficits observed in PON2-def mice [9].

Previous studies utilizing immortalized human renal proximal tubule (RPT) and HEK293 cells have shown PON2 to be involved in DA receptor signaling, with exposure to 1 μ M dopamine receptor agonist (DRD1/5 and DRD2/3) increasing PON2 expression [24, 25]. Although a higher concentration of 3 μ M was required to elicit an increase in PON2 expression in our study, our results in primary mouse neurons show that, like kidney cells, PON2 expression was increased upon exposure to

DRD2/3 agonist quinpirole. However, unlike kidney cells, no expression increase was observed upon exposure to DRD1/5 agonist fenoldopam. These data suggest there could be cell- and organ-specific differences in the role of PON2 in signaling. Conversely, these data may speak to important differences between immortalized versus primary cells, and/or species differences between humans and mice. The mechanism by which cells are immortalized involves disruption of normal cell proliferation, allowing cells to proliferate in perpetuity, which can alter their receptor function. As well, comparison of findings from different species can be difficult within the same cell type, much less across organ type. It would be of value to repeat our experimental conditions in primary mouse RPT cells to address organ-specific differences in PON2 DA signaling to eliminate many of these confounding factors.

No differences in DRD2 transcript or protein were observed in PON2-def mice compared to WT, supporting that PON2 is downstream of DRD2 and PON2 deficiency alone is not sufficient to alter DRD2 expression. This supports the existing literature examining PON2 levels in the kidney of DRD2 KO mice, where renal PON2 levels were 33% lower in DRD2 KO tissue compared to WT [24]. However, it is unclear whether PON2 plays a role in the presynaptic cell, postsynaptic cell, or both, as our methodology did not allow us to distinguish between DRD2 autoreceptor and postsynaptic receptor activation. Although the high concentration of quinpirole used in our study is expected to preferentially activate postsynaptic receptors, feasibly some autoreceptor activation is occurring, given the high affinity of DRD2 agonists for autoreceptors [50]. While the lack of significant PON2 modulation at a lower quinpirole concentration is encouraging that the observed increase is through postsynaptic binding, further exploration of the location of PON2 modulation would be of interest in future studies.

PON2 is primarily located to the mitochondrial membrane in the brain, although it has been additionally detected at the plasma membrane of neurons and astrocytes [6]. While the function at the plasma membrane is largely unknown, work in HEK 293 T cells has shown PON2 translocates to the plasma membrane under oxidative stress conditions to mitigate lipid peroxidation [51]. Although currently untested, PON2 may also translocate in neurons and astrocytes to interact with DRD2 at the synaptic membrane. Indeed, DRD2 and PON2 co-localize at the brush border of mouse renal proximal tubules [24], supporting extra-mitochondrial localization and direct DRD2 interaction in some cell types. Alternatively, PON2 may be upregulated in the mitochondria through DRD2 signaling cascades. In astrocytes, DRD2 has been found to modulate neuroinflammation by increased expression

of α B-crystallin (CRYAB) [52]. DRD2 null mice are reported to have an increased inflammatory response and dopaminergic neuron sensitivity to the neurotoxic compound MPTP [52], potentially owing to a decrease in CRYAB expression. CRYAB, a member of the heat shock protein family, primarily locates to the mitochondria where it exerts anti-inflammatory and anti-apoptotic action [53]. While no direct interactions between PON2 and CRYAB have been reported in the literature, this may be a further avenue of study going forward to determine if astrocytic modulation of neuroinflammation through the DRD2-CRYAB pathway involves PON2. Of additional interest would be examination of PON2 expression in subcellular fractions upon DRD2 agonist exposure to determine if the upregulation of PON2 is at the plasma membrane or at the mitochondria level, a limitation that was not addressed in our current study.

Conclusions

Evidence supports PON2 as a critical antioxidant enzyme in the brain, however, studies on its role in the CNS have been limited to date. Our study addresses key gaps in the literature regarding the effects of PON2 deficiency in the dopaminergic system of the CNS and demonstrates that PON2 deficiency significantly upregulates the transcript of multiple dopaminergic related genes in the striatum of mice. As well, our study identified potential organ-specific upregulation of PON2 through selective dopamine receptor signaling. Given the findings of this study, further investigation into PON2 in the dopaminergic system would be of interest to examine downstream consequences of these observed transcript changes and probe further into the role of PON2 in dopamine receptor signaling in the brain.

Abbreviations

CNS: Central nervous system; PON2: Paraoxonase 2; ROS: Reactive oxygen species; WT: Wildtype; PON2-def: Paraoxonase 2 deficient; DRD(1/2/3/5): Dopamine receptor (1/2/3/5); HDL: High density lipoproteins; DA: Dopamine; PD: Parkinson's disease; AD: Alzheimer's disease; 6-OHDA: 6-Hydroxydopamine; MPTP: 1-Methyl-4-phenyl-1,2,3,6-tetrahydropyridine; SNC: Substantia nigra pars compacta; PND: Postnatal day; CGN: Cerebellar granule neuron; TH: Tyrosine hydroxylase; VMAT2: Vesicular monoamine transporter 2; DAT: Dopamine transporter; HO-1/2: Heme-oxygenase 1/2; NOX2: NADPH oxidase 2; MAOA/B: Monoamine oxidase A/B; miRNA: Micro RNA; RPT: Renal proximal tubule; CRYAB: α B-crystallin.

Supplementary Information

The online version contains supplementary material available at <https://doi.org/10.1186/s12868-022-00738-4>.

Additional file 1. NormFinder results.

Additional file 2. Monoamine oxidase B (MAOB) results and western blot raw data.

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Author contributions

J.M.G. and K.D. performed experiments and analyzed the data. J.M.G., L.G.C., J.M. and C.E.F. contributed to the study conception and design. J.M.G. wrote the original manuscript. J.M.G., L.G.C., J.M. and C.E.F. revised the manuscript. L.G.C. and C.E.F. acquired financial support for the project. All authors read and approved the final manuscript.

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Availability of data and materials

All data generated and analyzed during the current study are available from the corresponding author on reasonable request.

Declarations

Ethics approval and consent to participate

All procedures were conducted in accordance with the National Institute of Health Guide for the Use and Care of Laboratory Animals and were approved by the University of Washington Institutional Animal Care and Use Committee. Animal numbers were kept to a minimum and all efforts were made to reduce animal suffering. All methods reported are reported in concurrence with ARRIVE guidelines for the reporting of animal experiments [54].

Consent for publication

Not applicable.

Competing interests

The authors declare no conflict of interest.

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References

1. Primo-Parmo SL, Sorenson RC, Teiber J, La Du B, N. The human serum paraoxonase/arylesterase gene (PON1) is one member of a multigene family. *Genomics*. 1996;33(3):498–507. <https://doi.org/10.1006/geno.1996.0225>.
2. Draganov DI, Teiber JF, Speelman A, Osawa Y, Sunahara R, La Du BN. Human paraoxonases (PON1, PON2, and PON3) are lactonases with overlapping and distinct substrate specificities. *J Lipid Res*. 2005;46(6):1239–47. <https://doi.org/10.1194/jlr.M400511-JLR200>.
3. Ng CJ, Wadleigh DJ, Gangopadhyay A, Hama S, Grijalva VR, Navab M, Fogelman AM, Reddy ST. Paraoxonase-2 is a ubiquitously expressed protein with antioxidant properties and is capable of preventing cell-mediated oxidative modification of low density lipoprotein. *J Biol Chem*. 2001;276(48):44444–9. <https://doi.org/10.1074/jbc.M105660200>.
4. Marsillach J, Mackness B, Mackness M, Riu F, Beltrán R, Joven J, Camps J. Immunohistochemical analysis of paraoxonases-1, 2, and 3 expression in normal mouse tissues. *Free Radic Biol Med*. 2008;45(2):146–57. <https://doi.org/10.1016/j.freeradbiomed.2008.03.023>.
5. Devarajan A, Bourquard N, Hama S, Navab M, Grijalva VR, Morvardi S, Clarke CF, Vergnes L, Reue K, Teiber JF, Reddy ST. Paraoxonase 2 deficiency alters mitochondrial function and exacerbates the development of atherosclerosis. *Antioxid Redox Signal*. 2011;14(3):341–51. <https://doi.org/10.1089/ars.2010.3430>.

6. Giordano G, Cole TB, Furlong CE, Costa LG. Paraoxonase 2 (PON2) in the mouse central nervous system: a neuroprotective role? *Toxicol Appl Pharmacol*. 2011;256(3):369–78. <https://doi.org/10.1016/j.taap.2011.02.014>.
7. Giordano G, Tait L, Furlong CE, Cole TB, Kavanagh TJ, Costa LG. Gender differences in brain susceptibility to oxidative stress are mediated by levels of paraoxonase-2 expression. *Free Radic Biol Med*. 2013;58:98–108. <https://doi.org/10.1016/j.freeradbiomed.2013.01.019>.
8. Jamwal S, Blackburn JK, Elsworth JD. Sex-based disparity in paraoxonase-2 expression in the brains of African green monkeys. *Free Radic Biol Med*. 2021;167:201–4. <https://doi.org/10.1016/j.freeradbiomed.2021.03.003>.
9. Garrick JM, Cole TB, Bammler TK, MacDonald JW, Marsillach J, Furlong CE, Costa LG. Paraoxonase 2 deficiency in mice alters motor behavior and causes region-specific transcript changes in the brain. *Neurotoxicol Teratol*. 2021;87: 107010. <https://doi.org/10.1016/j.ntt.2021.107010>.
10. Ng CJ, Bourquard N, Grijalva V, Hama S, Shih DM, Navab M, Fogelman AM, Lusic AJ, Young S, Reddy ST. Paraoxonase-2 deficiency aggravates atherosclerosis in mice despite lower apolipoprotein-B-containing lipoproteins: anti-atherogenic role for paraoxonase-2. *J Biol Chem*. 2006;281(40):29491–500. <https://doi.org/10.1074/jbc.M605379200>.
11. Li W, Kennedy D, Shao Z, Wang X, Kamdar AK, Weber M, Mislick K, Kiefer K, Morales R, Agatista-Boyle B, Shih DM, Reddy ST, Moravec CS, Tang WHW. Paraoxonase 2 prevents the development of heart failure. *Free Radic Biol Med*. 2018;121:117–26. <https://doi.org/10.1016/j.freeradbiomed.2018.04.583>.
12. Bourquard N, Ng CJ, Reddy ST. Impaired hepatic insulin signalling in PON2 deficient mice: a novel role for the PON2/apoE axis on the macrophage inflammatory response. *Biochem J*. 2011;436(1):91–100. <https://doi.org/10.1042/BJ20101891>.
13. Qujeq D, Mahrooz A, Alizadeh A, Boorank R. Paraoxonase-2 variants potentially influence insulin resistance, beta-cell function, and their interrelationships with alanine aminotransferase in type 2 diabetes. *J Res Med Sci*. 2018;23:107. https://doi.org/10.4103/jrms.JRMS_88_18.
14. Shih DM, Meng Y, Sallam T, Vergnes L, Shu ML, Reue K, Tontonoz P, Fogelman AM, Lusic AJ, Reddy ST. PON2 deficiency leads to increased susceptibility to diet-induced obesity. *Antioxidants (Basel, Switzerland)*. 2019. <https://doi.org/10.3390/antiox8010019>.
15. Nie Y, Luo D, Yang M, Wang Y, Xiong L, Gao L, Liu Y, Liu H. A meta-analysis on the relationship of the PON Genes and Alzheimer disease. *J Geriatr Psychiatry Neurol*. 2017;30(6):303–10. <https://doi.org/10.1177/0891988717731825>.
16. Altenhöfer S, Witte I, Teiber JF, Wilgenbus P, Pautz A, Li H, Daiber A, Witan H, Clement AM, Förstermann U, Horke S. One enzyme, two functions: PON2 prevents mitochondrial superoxide formation and apoptosis independent from its lactonase activity. *J Biol Chem*. 2010;285(32):24398–403. <https://doi.org/10.1074/jbc.M110.118604>.
17. Meiser J, Weindl D, Hiller K. Complexity of dopamine metabolism. *Cell Commun Signal*. 2013;11(1):34–34. <https://doi.org/10.1186/1478-811X-11-34>.
18. Wang X, Michaelis EK. Selective neuronal vulnerability to oxidative stress in the brain. *Front Aging Neurosci*. 2010;2:12–12. <https://doi.org/10.3389/fnagi.2010.00012>.
19. Blesa J, Trigo-Damas I, Quiroga-Varela A, Jackson-Lewis VR. Oxidative stress and Parkinson's disease. *Front Neuroanat*. 2015;9:91. <https://doi.org/10.3389/fnana.2015.00091>.
20. Khan Z, Ali SA. Oxidative stress-related biomarkers in Parkinson's disease: a systematic review and meta-analysis. *Iran J Neurol*. 2018;17(3):137–44.
21. Varçin M, Bentea E, Michotte Y, Sarre S. Oxidative stress in genetic mouse models of Parkinson's disease. *Oxid Med Cell Longev*. 2012;2012:624925–624925. <https://doi.org/10.1155/2012/624925>.
22. Parsanejad M, Bourquard N, Qu D, Zhang Y, Huang E, Rousseaux MWC, Aleyasin H, Irrcher I, Callaghan S, Vaillant DC, Kim RH, Slack RS, Mak TW, Reddy ST, Figeys D, Park DS. DJ-1 interacts with and regulates paraoxonase-2, an enzyme critical for neuronal survival in response to oxidative stress. *PLoS ONE*. 2014;9(9): e106601. <https://doi.org/10.1371/journal.pone.0106601>.
23. Harris RC, Zhang M-Z. Dopamine, the kidney, and hypertension. *Curr Hypertens Rep*. 2012;14(2):138–43. <https://doi.org/10.1007/s11906-012-0253-z>.
24. Yang Y, Zhang Y, Cuevas S, Villar VA, Escano C, Asico DL, Yu P, Grandy DK, Felder RA, Armando I, Jose PA. Paraoxonase 2 decreases renal reactive oxygen species production, lowers blood pressure, and mediates dopamine D2 receptor-induced inhibition of NADPH oxidase. *Free Radic Biol Med*. 2012;53(3):437–46. <https://doi.org/10.1016/j.freeradbiomed.2012.05.015>.
25. Yang S, Yang Y, Yu P, Yang J, Jiang X, Villar VAM, Sibley DR, Jose PA, Zeng C. Dopamine D1 and D5 receptors differentially regulate oxidative stress through paraoxonase 2 in kidney cells. *Free Radic Res*. 2015;49(4):397–410. <https://doi.org/10.3109/10715762.2015.1006215>.
26. Giordano G, White CC, McConnachie LA, Fernandez C, Kavanagh TJ, Costa LG. Neurotoxicity of domoic acid in cerebellar granule neurons in a genetic model of glutathione deficiency. *Mol Pharmacol*. 2006;70(6):2116. <https://doi.org/10.1124/mol.106.027748>.
27. Frantz KJ, Van Hartesveldt C. Sulpiride antagonizes the biphasic locomotor effects of quinpirole in weanling rats. *Psychopharmacology*. 1995;119(3):299–304. <https://doi.org/10.1007/BF02246295>.
28. Shi WX, Smith PL, Pun CL, Millet B, Bunney BS. D1–D2 interaction in feedback control of midbrain dopamine neurons. *J Neurosci*. 1997;17(20):7988–94. <https://doi.org/10.1523/JNEUROSCI.17-20-07988.1997>.
29. Garrick JM, Dao K, de Laat R, Elsworth J, Cole TB, Marsillach J, Furlong CE, Costa LG. Developmental expression of paraoxonase 2. *Chem Biol Interact*. 2016;259(Pt B):168–74. <https://doi.org/10.1016/j.cbi.2016.04.001>.
30. Andersen CL, Jensen JL, Ørntoft TF. Normalization of real-time quantitative reverse transcription-PCR data: a model-based variance estimation approach to identify genes suited for normalization, applied to bladder and colon cancer data sets. *Can Res*. 2004;64(15):5245–50. <https://doi.org/10.1158/0008-5472.CAN-04-0496>.
31. Dasgupta S, Demirci FY, Dressen AS, Kao AH, Rhew EY, Ramsey-Goldman R, Manzi S, Kammerer CM, Kamboh MI. Association analysis of PON2 genetic variants with serum paraoxonase activity and systemic lupus erythematosus. *BMC Med Genet*. 2011;12:7. <https://doi.org/10.1186/1471-2350-12-7>.
32. Wachtel SR, Abercrombie ED. L-3,4-dihydroxyphenylalanine-induced dopamine release in the striatum of intact and 6-hydroxydopamine-treated rats: differential effects of monoamine oxidase A and B inhibitors. *J Neurochem*. 1994;63(1):108–17. <https://doi.org/10.1046/j.1471-4159.1994.63010108.x>.
33. Fornai F, Chen K, Giorgi FS, Gesi M, Alessandri MG, Shih JC. Striatal dopamine metabolism in monoamine oxidase B-deficient mice: a brain dialysis study. *J Neurochem*. 1999;73(6):2434–40. <https://doi.org/10.1046/j.1471-4159.1999.0732434.x>.
34. Maier T, Güell M, Serrano L. Correlation of mRNA and protein in complex biological samples. *FEBS Lett*. 2009;583(24):3966–73. <https://doi.org/10.1016/j.febslet.2009.10.036>.
35. Carlyle BC, Kitchen RR, Kanyo JE, Voss EZ, Pletikos M, Sousa A, Lam TT, Gerstein MB, Sestan N, Nairn AC. A multiregional proteomic survey of the postnatal human brain. *Nat Neurosci*. 2017;20(12):1787–95. <https://doi.org/10.1038/s41593-017-0011-2>.
36. Holt CE, Schuman EM. The central dogma decentralized: new perspectives on RNA function and local translation in neurons. *Neuron*. 2013;80(3):648–57. <https://doi.org/10.1016/j.neuron.2013.10.036>.
37. Crispino M, Chun JT, Cefaliello C, Perrone Capano C, Giuditta A. Local gene expression in nerve endings. *Dev Neurobiol*. 2014;74(3):279–91. <https://doi.org/10.1002/dneu.22109>.
38. Gervasi NM, Scott SS, Aschrafi A, Gale J, Vohra SN, MacGibeny MA, Kar AN, Gioio AE, Kaplan BB. The local expression and trafficking of tyrosine hydroxylase mRNA in the axons of sympathetic neurons. *RNA (New York, NY)*. 2016;22(6):883–95. <https://doi.org/10.1261/rna.053272.115>.
39. Xenias HS, Ibáñez-Sandoval O, Koós T, Tepper JM. Are striatal tyrosine hydroxylase interneurons dopaminergic? *J Neurosci*. 2015;35(16):6584–99. <https://doi.org/10.1523/JNEUROSCI.0195-15.2015>.
40. Marin O, Anderson SA, Rubenstein JL. Origin and molecular specification of striatal interneurons. *J Neurosci*. 2000;20(16):6063–76. <https://doi.org/10.1523/JNEUROSCI.20-16-06063.2000>.
41. Liu Y, Beyer A, Aebersold R. On the dependency of cellular protein levels on mRNA abundance. *Cell*. 2016;165(3):535–50. <https://doi.org/10.1016/j.cell.2016.03.014>.
42. Hershey JWB, Sonenberg N, Mathews MB. Principles of translational control: an overview. *Cold Spring Harb Perspect Biol*. 2012;4(12): a011528. <https://doi.org/10.1101/cshperspect.a011528>.

43. Cooper GM. Protein degradation. The cell: a molecular approach. 2nd edn. 2000. <https://www.ncbi.nlm.nih.gov/books/NBK9957/>
44. Anisimova AS, Alexandrov AI, Makarova NE, Gladyshev VN, Dmitriev SE. Protein synthesis and quality control in aging. *Aging*. 2018;10(12):4269–88. <https://doi.org/10.18632/aging.101721>.
45. Kellendonk C, Simpson EH, Polan HJ, Malleret G, Vronskaya S, Winiger V, Moore H, Kandel ER. Transient and selective overexpression of dopamine D2 receptors in the striatum causes persistent abnormalities in prefrontal cortex functioning. *Neuron*. 2006;49(4):603–15. <https://doi.org/10.1016/j.neuron.2006.01.023>.
46. Huang W-J, Zhang X, Chen W-W. Role of oxidative stress in Alzheimer's disease. *Biomed Rep*. 2016;4(5):519–22. <https://doi.org/10.3892/br.2016.630>.
47. Boyd KN, Mailman RB. Dopamine receptor signaling and current and future antipsychotic drugs. *Handb Exp Pharmacol*. 2012;212:53. https://doi.org/10.1007/978-3-642-25761-2_3.
48. Mishra A, Singh S, Shukla S. Physiological and functional basis of dopamine receptors and their role in neurogenesis: possible implication for Parkinson's disease. *J Exp Neurosci*. 2018. <https://doi.org/10.1177/1179069518779829>.
49. Kelly MA, Rubinstein M, Phillips TJ, Lessov CN, Burkhart-Kasch S, Zhang G, Bunzow JR, Fang Y, Gerhardt GA, Grandy DK, Low MJ. Locomotor activity in D2 dopamine receptor-deficient mice is determined by gene dosage, genetic background, and developmental adaptations. *J Neurosci*. 1998;18(9):3470. <https://doi.org/10.1523/JNEUROSCI.18-09-03470.1998>.
50. Ford CP. The role of D2-autoreceptors in regulating dopamine neuron activity and transmission. *Neuroscience*. 2014;282:13–22. <https://doi.org/10.1016/j.neuroscience.2014.01.025>.
51. Hagmann H, Kuczkowski A, Ruehl M, Lamkemeyer T, Brodesser S, Horke S, Dryer S, Schermer B, Benzing T, Brinkkoetter PT. Breaking the chain at the membrane: paraoxonase 2 counteracts lipid peroxidation at the plasma membrane. *FASEB J*. 2014;28(4):1769–79. <https://doi.org/10.1096/fj.13-240309>.
52. Shao W, Zhang S, Tang M, Zhang X, Zhou Z, Yin Y, Zhou Q, Huang Y, Liu Y, Wawrousek E, Chen T, Li S, Xu M, Zhou J, Hu G, Zhou J. Suppression of neuroinflammation by astrocytic dopamine D2 receptors via α B-crystallin. *Nature*. 2013;494(7435):90–4. <https://doi.org/10.1038/nature11748>.
53. Ousman SS, Tomooka BH, van Noort JM, Wawrousek EF, O'Connor KC, Hafler DA, Sobel RA, Robinson WH, Steinman L. Protective and therapeutic role for α B-crystallin in autoimmune demyelination. *Nature*. 2007;448(7152):474–9. <https://doi.org/10.1038/nature05935>.
54. du Sert NP, Hurst V, Ahluwalia A, Alam S, Avey MT, Baker M, Browne WJ, Clark A, Cuthill IC, Dirnagl U, Emerson M, Garner P, Holgate ST, Howells DW, Karp NA, Lazic SE, Lidster K, MacCallum CJ, Macleod M, et al. The ARRIVE guidelines 2.0: updated guidelines for reporting animal research. *PLoS Biol*. 2020;18(7): e3000410. <https://doi.org/10.1371/journal.pbio.3000410>.

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