Dopaminergic Positron Emission Tomography Imaging in the Alpha-Synuclein Preformed Fibril Model Reveals Similarities to Early Parkinson’s Disease

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ABSTRACT: Background: Positron emission tomography (PET) imaging in early Parkinson’s disease (PD) subjects reveals that increased dopamine (DA) turnover and reduced dopamine transporter (DAT) density precede decreases in DA synthesis and storage. The rat α-synuclein preformed fibril (α-syn PFF) model provides a platform to investigate DA dynamics during multiple stages of α-syn inclusion-triggered nigrostriatal degeneration.

Objectives: We investigated multiple aspects of in vivo dopaminergic deficits longitudinally and similarities to human PD using translational PET imaging readouts.

Methods: Longitudinal imaging was performed every 2 months in PFF and control rats for 7 months. [18F]-Fluoro-3,4-dihydroxyphenyl-L-alanine (FDOPA) imaging was performed to investigate DA synthesis and storage (Kocc) and DA turnover, estimated by its inverse, the effective distribution volume ratio (EDVR). [11C]-Methylphenidate (MP) was used to estimate DAT density (BPND).

Results: Early DA turnover increases and DAT binding decreases were observed in the ipsilateral striatum of PFF rats, progressing longitudinally. EDVR decreased 26%, 38%, and 47%, and BPND decreased 36%, 50%, and 65% at the 2-, 4-, and 6-month time points, respectively, compared to ipsilateral control striatum. In contrast, deficits in DA synthesis and storage were not observed in the ipsilateral striatum of PFF rats compared to control injections and were relatively preserved up to 6 months (Kocc decreased 20% at 6 months).

Conclusions: The relative preservation of DA synthesis and storage compared to robust progressive deficits in DAT density and increases in DA turnover in the rat α-syn PFF model display remarkable face validity to dopaminergic alterations in human PD. © 2022 The Authors. Movement Disorders published by Wiley Periodicals LLC on behalf of International Parkinson and Movement Disorder Society

Key Words: alpha-synuclein; synucleinopathy; rodent model; PET imaging; nigrostriatal system

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Parkinson’s disease (PD) is characterized by the degeneration of the nigrostriatal system within the context of accumulation of pathological alpha-synuclein (α-syn) inclusions (Lewy bodies and Lewy neurites). Using [18F]-fluoro-3,4-dihydroxyphenyl-L-alanine (FDOPA) positron emission tomography (PET) imaging we have shown that increased dopamine (DA) turnover, estimated as reduction of its inverse, the effective dopamine distribution volume (EDV), precedes decreases in DA synthesis and storage in early PD.¹ Similarly, asymptomatic carriers of leucine-rich repeat kinase 2 (LRRK2) exhibit normal DA synthesis and storage; however, DA turnover is increased and dopamine transporter (DAT; [11C]-methylphenidate [MP])
and vesicular monoamine transporter type 2 (VMAT2; $^{11}$C-($\alpha$)-dihydrotetrabenazine [DTBZ]) are reduced.$^{2,3}$ In general, as the disease progresses, deficits in DA synthesis and storage remain relatively modest compared to other dopaminergic PET markers.$^{4,5}$ These findings underscore that nigrostriatal degeneration in PD is dynamic, with disease stage-dependent events impacting dopaminergic transmission. Understanding the DA dynamics of PD progression will inform the development of dopaminergic therapies.

PET imaging has been applied to neurotoxicant and $\alpha$-syn overexpression rodent models that result in rapid, severe degeneration of the nigrostriatal system.$^{6-10}$ In general, these studies demonstrate that decreased DAT, VMAT2, DA synthesis and storage, and DA turnover mirror lesion status as defined by histological markers of nigrostriatal neuron survival. However, none of these models recapitulate the prolonged stage of Lewy body (LB) accumulation associated with PD and thus cannot inform the impact of this phenomenon on DA dynamics. Recently, we have characterized the impact of intrastriatal injection of preformed $\alpha$-syn fibrils into rats.$^{11-14}$ In this model, intraneuronal LB-like phosphorylated $\alpha$-syn inclusions are triggered to form within structures innervating the striatum, including the nigrostriatal system. Nigral $\alpha$-syn inclusion formation peaks 2 months after injection, with significant loss of dopaminergic phenotype observed at 4 months and overt degeneration occurring 5–6 months following injection.$^{12,13}$ Thus, the rat $\alpha$-syn PFF model provides a platform to investigate DA dynamics during multiple stages of $\alpha$-syn inclusion-triggered nigrostriatal degeneration. In the present study we used longitudinal multitracer PET imaging to investigate DA synthesis and storage, DA turnover, and DAT binding in the rat $\alpha$-syn PFF model. Our results indicate that the degenerative cascade of events triggered by intrastriatal PFF injections into rats results in progression of dopaminergic dysfunction similar to that observed in early PD.

**Methods**

Rats were unilaterally injected with either preformed $\alpha$-syn fibrils (PFF, $n = 8$) or Dulbecco’s phosphate buffered saline (PBS) (control, $n = 8$) into two sites in the dorsal striatum. PET scans with $^{18}$F-FDOPA (effective distribution volume ratio [EDVR, the tissue input equivalent of EDV]$^{7,15}$ for turnover and $K_{acc}$ for DA synthesis)$^{1}$ and $^{11}$C-MP (BP$_{ND}$) were performed at 2, 4, and 6 months (PFF rats) or 3, 5, or 7 months (control). At the conclusion of PET scanning all rats were perfused intracardially and brains analyzed at 7.5 months following surgery. Fig. 1A illustrates the experimental design.

**Rats**

Male, 3-month-old Fischer 344 rats (Charles River, Wilmington, MA) were housed in the Association for Assessment and Accreditation of Laboratory Animal Care (AAALAC) -approved MSU Grand Rapids Research Center vivarium and the UBC Animal Resource Unit. All procedures were conducted in accordance with guidelines set by the Institutional Animal Care and Use Committee (IACUC) of MSU and the UBC Animal Care Committee.

**Preparation and Intrastriatal Injection of $\alpha$-syn PFFs**

Recombinant, full-length mouse $\alpha$-syn PFFs were prepared.$^{16,17}$ Mouse $\alpha$-syn was selected to generate PFFs due to the superior efficiency of this protein species in seeding pathological inclusions in rodents.$^{18,19}$ Prior to injection, PFFs were thawed, diluted to (4 $\mu$g/$\mu$L), and sonicated at room temperature. A sample of the PFFs was imaged on a JEOL JEM-1400 transmission electron microscope. The length of ~500 fibrils per sample was measured to validate that average fibril size approximated 50 nm (Fig. 1B). Intrastriatal $\alpha$-syn PFF injections were conducted as described previously.$^{12,14,16}$ Rats were anesthetized before surgery with isoflurane and each rat received two unilateral, intrastriatal injections (AP $+1.0$ mm, ML $+2.0$ mm, DV $-4.0$ mm and AP $+0.1$ mm, ML $+4.2$ mm, DV $-5.0$ mm, AP and ML relative to bregma and DV relative to dura, injection rate 0.5 $\mu$L/min.) of sonicated $\alpha$-syn PFFs (4 $\mu$g/$\mu$L, 2 $\times$ 2 $\mu$L injections) or an equal volume of Dulbecco’s PBS.

**Scanning**

The injected activity, administered as bolus, was $4.5 \pm 0.2$ MBq (mean ± SD) per 100 g of body weight for $^{18}$F-fluoro-3,4-dihydroxyphenyl-L-alanine (FDOPA)$^{20}$ and $4.4 \pm 0.2$ MBq per 100 g of body weight for $^{11}$C-methylphenidate (MP).$^{21}$ The specific activity at injection was $18 \pm 10$ MBq/micromole (mean ± SD) for FDOPA and $\geq 150$ GBq/micromole for MP.

Rats were scanned with both tracers at 2, 4, and 6 months after surgery (PFF rats) or 3, 5, or 7 months after surgery (control rats). A minimum of 5 days was allowed between two subsequent scans on the same animal to allow for full recovery between scans (with the exception of two animals at a single time point due to technical reasons). All studies were performed on a Siemens/Concorde microPET Focus 120.

The scanning procedure was similar for both tracers: the rats were anesthetized and maintained with a 2% isoflurane/O$_2$ gas mixture. Each rat was positioned in a stereotactic head-holder mounted to the scanner bed, allowing accurate and reproducible positioning. After a 6-min transmission scan with a $^{57}$Co source, emission scans were acquired dynamically starting at injection. MP data were
acquired over 61 min and framed into 17 frames (6 × 30 s; 2 × 60 s; 5 × 300 s; 2 × 450 s; and 2 × 480 s). Ninety minutes prior to FDOPA injection, entacapone (20 mg/kg), a catechol-O-methyltransferase (COMT) inhibitor, was administered intraperitoneally (IP), followed by IP administration of the aromatic L-amino acid decarboxylase (AADC) inhibitor benseraside (10 mg/kg) 30 min prior to tracer injection to minimize peripheral FDOPA metabolism. Data were acquired for 150 min and framed into 24 frames (6 × 30 s; 2 × 60 s; 5 × 300 s; 3 × 400 s; 6 × 700 s; and 2 × 900 s). Heart rate, blood oxygen saturation level, and temperature were continuously monitored to ensure normal range. Details and validation of the FDOPA imaging and analysis are as published previously.⁶,⁷

### Image Analysis

Time–activity curves (TACs) were extracted from the images using predefined regions of interest (ROIs) using the ASIPro software (CTI Concorde Microsystems, Knoxville, TN). For each scan, ROIs were placed on each striatum and on the cerebellum, used as reference region, on the three axial planes where the structures were visually best defined. The ROI volumes were 0.043 cm³ (cerebellum) and 0.022 cm³ (striatal). The TACs from the FDOPA images were used to estimate $K_{occ}$ and EDVR with in-house software using Matlab (The Mathworks, Natick, MA). The tissue input Logan graphical approach was used to estimate MP $BP_{ND}$, a measure of DAT density. 

### Immunohistochemistry and Unbiased Stereology

Seven months following surgery all rats were deeply anesthetized and perfused intracardially with heparinized normal saline followed by 4% paraformaldehyde. Free-floating brain sections (40 μm) were immunolabeled with primary antibodies: mouse anti-phosphorylated α-syn at serine 129 (pSyn, 81A; Abcam, Cambridge, MA; AB184674; 1:10000), rabbit anti-tyrosine hydroxylase (TH; Millipore, Temecula, CA; AB152, 1:4000), or mouse anti HuC (Invitrogen, Wal- tham, MA; A-21271, 1:2000) followed by the appropriate biotinylated secondary antibodies, amplified and visualized by development in 3,3′-diaminobenzidine (DAB; Sigma-Aldrich, St. Louis, MO; D5637-10G) and 0.03% H₂O₂. The number of tyrosine hydroxylase immunoreactive (THir) and HuC immunoreactive neurons (HuCir; pan neuronal marker; L-AADC, aromatic L-amino acid decarboxylase; MAO, monoamine oxidase; COMT, catechol-O-methyltransferase. [Color figure can be viewed at wileyonlinelibrary.com]
(SNpc) was estimated using unbiased stereology with the optical fractionator principle.\textsuperscript{12,13}

**Immunofluorescence**

Striatal sections were immunolabeled using rabbit anti-dopamine transporter (DAT; Millipore, Temecula, CA; D6944, 1:1000) followed by secondary labeling with near-infrared secondary antibody (donkey anti-rabbit 800CW IRDye, LiCor #926-32213, 1:500) and imaged on a LiCor Odyssey CLx flatbed scanner as described previously.\textsuperscript{12,22} The near-infrared immunofluorescent intensity signal for DAT was quantified in every sixth 40 μM coronal section through the caudate-putamen.

**Statistical Analysis**

Statistical tests were completed using either GraphPad Prism software (version 9, GraphPad, La Jolla, CA) or in R (version 4.0.3) using the \textit{lme4} package. Two-way analysis of variance (ANOVA) with repeated measures was used to determine significant differences between the injected hemispheres or between the uninjected hemispheres, or between time points within hemispheres of PFF- or PBS-injected rats, using Sidak’s multiple comparison tests. Simple linear regression modeling was used to examine relationships at individual time points whereas a linear mixed effects regression model was used to examine relationships of repeated measures across multiple time points.

**Results**

**Impact of PFF-Induced Pathology on DA Synthesis and Storage is Relatively Modest and Delayed**

Rats were imaged with FDOPA to estimate the DA synthesis and storage uptake rate \((K_{occ})\) in both the contralateral un.injected striatum and ipsilateral striatum that received PFF injection. In the un injected striatum of either PFF or control rats, no significant effect of treatment or postsurgical interval on \(K_{occ}\) was observed \((P > 0.05); \text{Fig. 2A}\). In order to control for the effect of intrastriatal surgical injection artifact, primary comparisons were made between the ipsilateral striata of control- and PFF-injected rats (\text{Fig. 2B}). No significant differences in \(K_{occ}\) were observed between the ipsilateral striatum of PFF and control injected rats at any of the time points \((P > 0.05)\). Within the PFF-injected striatum a significant decrease of ~20–27% \(K_{occ}\) was observed over time (4–6 months) compared to the 2-month time point \((P < 0.05)\), with no progression observed between 4 and 6 months. These results suggest that DA synthesis and storage is modestly impacted by PFF injection-associated toxicity, and only at relatively later time points.

**PFF Injection-Induced Degeneration Impacts DA Turnover earlier and to a Greater Degree than DA Synthesis and Storage**

FDOPA data were also evaluated for the effective dopamine distribution volume ratio (EDVR) (\text{Fig. 1C}); a decrease in EDVR reflects increased DA turnover.\textsuperscript{1} In the un injected striatum of either PFF or control rats, no significant effect of treatment or postsurgical interval on EDVR was observed \((P > 0.05); \text{Fig. 2C})

However, a significant reduction of EDVR was observed in the ipsilateral striatum of PFF-injected rats compared to control injections at all time points examined (\text{Fig. 2D;} \(P < 0.01\)). Specifically, we observed a ~26–38%, and ~47% reduction at the 2-, 4-, and 6-month time points, respectively. Repeated measures analysis revealed that the PFF-associated decrease of EDVR progressed significantly between 2 months and 4 months following injection \((P < 0.05)\), with PFF rats at both 4 and 6 months exhibiting significantly lower EDVR than at 2 months \((P < 0.05)\). Collectively, these results suggest that the pathology produced by PFF injection results in early and progressive increases in DA turnover following PFF injection.

**PFF Injection-Induced Pathology Results in Early and Progressive Reduction in DAT Density in the Ipsilateral Striatum**

Using \(^{11}\text{C}\)-methylphenidate (MP) we examined DAT density rats across a 7-month time span. In the uninjected striatum of either PFF or control rats, no significant effect of treatment or postsurgical interval on BP\(_{ND}\) was observed \((P > 0.05); \text{Fig. 2E})

However, comparisons between the ipsilateral striata of control and PFF-injected rats (\text{Fig. 2F}) revealed that PFF injection resulted in a significant reduction in BP\(_{ND}\) compared to control injections at all time points \((P < 0.0001)\). Specifically, we observed a ~36% and ~50% reduction in BP\(_{ND}\) at the 2- and 4-month time points, progressing to ~65% at the 6-month time point (\text{Fig. 2F,G}). Repeated measures analysis revealed a significant progressive decrease of BP\(_{ND}\) in the ipsilateral striatum of PFF rats over time, with PFF rats at 4 months exhibiting significantly lower BP\(_{ND}\) than at 2 months \((P < 0.001)\) and PFF rats at 6 months significantly lower than at 4 months \((P < 0.05)\). Collectively, these results demonstrate that the synucleinopathy-induced degeneration following intrastriatal PFF injection results in significant and progressive loss of DAT binding in the ipsilateral striatum.

**Decreased DAT and Enhanced DA Turnover Induced by PFF Injection-Induced Pathology are Significantly Associated**

We next examined the relationship between DAT (MP BP\(_{ND}\)) and DA turnover ratio (EDVR) and
between DAT and DA synthesis/storage ($K_{occ}$) within individual rats. In control rats, no significant relationship was observed in either hemisphere at any time point ($P > 0.05$). In contrast, we observed a significant relationship between MP BPND and EDVR within the ipsilateral striatum of control-injected rats. Within the PFF-injected striatum, $K_{occ}$ was significantly decreased at the 4 and 6 month time points compared to the 2-month time point ($P > 0.05$). In contrast, we observed a significant relationship between MP BPND and EDVR within the contralateral hemisphere of PFF-injected rats at 6 months ($P < 0.05$, $R^2 = 0.50$; Fig. 3A). We also observed a significant relationship between MP BPND and EDVR within the contralateral hemisphere of PFF-injected rats at 6 months ($P < 0.05$, $R^2 = 0.50$; Fig. 3A). No significant relationship was observed in either hemisphere at any specific time point ($P > 0.05$; Fig. 3B) or when examined across all time points ($P > 0.05$, data not shown). Thus, we observed that in addition to the ipsilateral striatum of PFF-injected rats exhibiting reduced DAT and increased DA turnover relative to the contralateral hemisphere, within the ipsilateral striatum at 2 and 6 months decreased DAT was associated with increased DA turnover. Further, within the contralateral striatum of PFF-injected rats at 6 months, decreased DAT also correlated with increased DA turnover.

**DAT Immunofluorescence Intensity is Significantly Reduced in the Ipsilateral Striatum 7 Months after PFF Injection**

After completion of the longitudinal study, rats were analyzed for immunofluorescent DAT intensity in the striatum. Decreased DAT signal spanned the striatum ipsilateral to PFF injection in all rats with no alterations in DAT signal observed ipsilateral to PBS injection (Fig. 4A). Quantification revealed that PFF injection resulted in a significant ~58% reduction in DAT immunofluorescence compared to control (Fig. 4B; $P < 0.01$).
This reduction was similar to the ~65% reduction in MP BP
ND in the ipsilateral striatum of the PFF-injected rats observed at the 6-month time point (Fig. 2F). To examine the relationship between in vivo PET data and post-mortem immunofluorescent analyses, we compared striatal DAT immunofluorescence intensity at 7 months to MP BP
ND-derived estimates of DAT density for the ipsilateral striatum of the PFF-injected rats at 6 months and that obtained for PBS-injected rats at 7 months. A strong, significant correlation (Fig. 4C; R^2 = 0.8251, P < 0.0001) was found. These results suggest that post-mortem immunofluorescent labeling of DAT agrees well with the BP
ND of MP.

**Intrastriatal PFF Injection Resulted in Accumulation of Phosphorylated α-Syn (pSyn) Inclusions**

All rats injected with PFFs exhibited numerous inclusions immunoreactive for phosphorylated α-syn (pSyn) within neurons in various cortical regions and the striatum (Fig. 4D,E). Some pSyn inclusions also were observed in the SNpc ipsilateral to α-syn PFF injection (Fig. 4F). No pSyn inclusions were observed in control rats. This pattern of pSyn accumulation replicates what we have previously observed 6 months following intrastriatal PFF injections to rats in which pSyn inclusion formation in the SNpc and cortex peaks at earlier time points (1–2 months); pathology is maintained in the cortex over time but decreases in the SNpc as pSyn-containing nigrostriatal DA neurons degenerate. In addition, accumulation of pSyn-ir neurons in the striatum peaks at later intervals after intrastriatal PFF injection at approximately 6 months and was present in abundance at the 7-month time point.

**Intrastriatal PFF Injection Results in Marked Nigrostriatal Degeneration in the Ipsilateral Hemisphere**

Examination of DAT immunoreactivity in the striatum revealed a marked denervation in the ipsilateral striatum in PFF-injected rats unlike in the ipsilateral striatum of controls (Fig. 4A,B). Similarly, in PFF-injected rats a marked loss (~60%) of SNpc THir neurons was observed ipsilaterally (P < 0.0001; Fig. 4H) with no loss observed in the ipsilateral SNpc of control rats (P > 0.05; Fig. 4H). Stereological quantification of HuCir neurons revealed a significant reduction in neurons in the ipsilateral SNpc of PFF-injected rats (P < 0.0001; Fig. 4I) with no loss of SNpc neurons observed in controls (P > 0.05; Fig. 4I). The average total number of SNpc THir neurons lost (compared to the contralateral hemisphere) in PFF-injected rats was 10,450 compared to 9450 HuCir neurons lost, indicating that the overwhelming majority of loss represented overt neuronal degeneration and not simply a downregulation of TH phenotype.

**Discussion**

Previously, we have demonstrated that intrastriatal injection of α-syn PFFs to rats results in peak...
phosphorylated α-syn (pSyn) inclusion formation in the SNpc at 2 months, followed by loss of TH phenotype and ultimately nigrostriatal degeneration between 4 and 6 months. The later degenerative phase is also associated with significant deficits in motor function and striatal DA. In the present study we used multitracer PET imaging to address two different questions: (i) Are other aspects of dopaminergic alterations...
detectable in the α-syn PFF rat model? and (ii) Do their relative magnitude and time courses compare to what is observed in early human PD? We used an imaging-based readout similar to that which we previously used in sporadic and LRRK2 mutation-related human PD. Overall, a striking similarity between the human PD studies and the PFF-induced synucleinopathy model was observed. The PFF injection resulted in an early reduction in DAT binding that progressed significantly over the 6-month study interval. This decrease in DAT was paralleled by a significant and progressive increase in DA turnover (decrease in EDVR); whereas DA synthesis and storage exhibited later and modest reductions that remained relatively stable over time. Similarly, early PD subjects, as well as asymptomatic LRRK2 mutation carriers, exhibit increased DA turnover and decreased DAT with relatively preserved DA synthesis and storage. Our human data led to the interpretation that a functional role of DAT is to maintain relatively constant synaptic DA levels and to preserve DA in nerve terminals and that clinical motor symptoms manifest when the surviving neurons become unable to maintain levels of DA synthesis and storage; at that time Kocc decreases by approximately 30–40% with DAT binding decreasing by ~55–60%. In the present α-syn PFF model study, we did not observe a decrease in Kocc until 4 months after PFF injection, with the reduction in Kocc relatively smaller compared to reductions in DAT binding and increased DA turnover (20–27% reduction in Kocc compared to ~65% and ~47% reduction in DAT binding and EDVR, respectively). In the context of human disease, it is thus not completely surprising that the PFF-injected rats do not exhibit observable motor deficits until 6 months after injection. Overall, our rat α-syn PFF model study indicates that the pattern of dopaminergic dysfunction resulting from PFF-triggered nigrostriatal synucleinopathy accurately recapitulates the progression of dopaminergic dysfunction observed in early PD.

The specific pathogenic relationship between intraneuronal pSyn immunoreactive inclusions and the ultimate degeneration of the nigrostriatal system remains unclear. pSyn inclusions colocalize with markers commonly observed in LB (p62 and ubiquitin), consist of α-syn oligomers and fibrils, and are also thioflavin-S positive and proteinase-K resistant. Inclusion-bearing neurons selectively degenerate in the α-syn PFF model; however, the mechanistic link between the presence of pSyn immunoreactive aggregates and neuronal toxicity remains unknown. α-Syn aggregates and/or oligomers may be directly toxic, depletion of monomeric α-syn may have toxic consequences, and/or other mechanisms not yet fully understood may contribute to degeneration of inclusion-bearing neurons. Thus, in the present study, pSyn immunoreactivity in the SNpc predicts, but does not necessarily directly cause, pathogenic consequences. Previous studies have used PET imaging of DAT, vesicular monoamine transporter 2 (VMAT2), DA synthesis/storage, and DA turnover in either the 6-hydroxydopamine (6-OHDA) or viral vector-mediated α-syn overexpression model in rats. In all of these studies severe loss of nigrostriatal neurons was documented within 1–2 weeks post-lesion, with PET imaging tracking with lesion severity. The rat α-syn PFF model offers the ability to examine DA dynamics over a protracted interval when nigrostriatal DA neurons remain alive but increasingly more dysfunctional. Using model parameters identical to the present study, we observe peak α-syn inclusion formation in nigral neurons for a period of 2 months after PFF injection with SNpc THir neurons maintained to near control levels. At 4 months after PFF injection significant loss of TH phenotype is observed; however, no significant degeneration of SNpc (neuronal loss) is observed until at least 5 months. Thus, the α-syn PFF model provides insight into the DA dynamics of inclusion-bearing nigrostriatal neurons at distinct phases of degeneration. At the 2-month time point we observe that α-syn inclusion-bearing SNpc neurons maintain DA synthesis and storage; however, DA turnover is increased and DAT density is reduced. At this same time point we have recently observed a significant reduction in DAT mRNA in the pSyn inclusion-seeded SNpc. The loss of MP BPND at the 2-month time point might therefore reflect either a loss of DA terminal density and/or a loss of DAT density in the absence of terminal loss. α-Syn and DAT are known protein partners, cell surface recruitment of DAT is modulated by α-syn, serine 129 phosphorylation of α-syn reduces normal facilitation of DAT function, and in the PD striatum DAT/α-syn complexes are redistributed and reduced. At the 4-month time point, when significant loss of TH phenotype is observed, DA synthesis and storage becomes compromised, DA turnover increases further, and DAT is further reduced. Finally, at the 6-month time point, increased DA turnover is maintained, DAT decreases further, and DA synthesis and storage remains only modestly impacted (Fig. 5). In the only other study to date that applied PET imaging to the rat α-syn PFF model, VMAT2 ([11C]-dihydroxytetrabenazine) was significantly reduced compared to control-injected striatum 4 months after PFF injections. The slower time course of VMAT2 reduction likely reflects the lower quantities of PFFs used compared to that which we used in the present study, resulting in a less efficient seeding of pSyn inclusions. No additional dopaminergic imaging measures were included in the Thomsen et al 2021 report. Thus, through simultaneous incorporation of Kocc, EDVR, and MP imaging, our study is the first to provide...
FIG. 5. Time course of dopaminergic alterations in the rat α-synuclein preformed fibril (PFF) model as revealed by immunohistochemistry and postmortem emission tomography imaging. Changes in striatal tyrosine hydroxylase (TH) immunoreactivity, substantia nigra pars compacta (SN) TH neuron number, and total SN neuron number are based on previous studies using the same intrastriatal α-syn PFF parameters at 2, 4, and 6 months after surgery.12 Two months: intrastriatal α-syn PFF injection results in little to no change in striatal TH immunoreactivity, SN TH immunoreactive (THir) neurons, or dopamine (DA) synthesis or storage; however, striatal dopamine transporter (DAT) binding is significantly reduced and DA turnover is significantly increased. This time point corresponds to peak accumulation of pathological α-syn inclusions in the SN.12-14 Four months: decreased striatal TH, reductions in SN THir neurons, and reduced DA synthesis and storage are detectable for the first time. SN neurons have lost TH phenotype but have not yet degenerated. Reductions in striatal DAT binding and increased DA turnover progress further. Six months: further loss of SN THir neurons, now representing overt degeneration. Maintained reductions in striatal TH immunoreactivity and increases in DA turnover. Modest reductions in striatal DA synthesis and storage are maintained, and reductions in striatal DAT binding progress further.

Data Availability Statement

The data that support the findings of this study are available from the corresponding author upon reasonable request.

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a progression of dopaminergic dysfunction similar to that observed in early PD. The formation of LB-like α-syn inclusions in nigrostriatal DA neurons is associated with decreased DAT density and an increase in DA turnover in the striatum. The progression of inclusion-seeded nigrostriatal neurons to ultimate degeneration is associated with a decrease in DA synthesis and storage in the striatum; however, this is relatively spared earlier in the time course of the lesion. The face validity of the rat α-syn PFF model provides a clinically relevant platform to investigate the early consequences of pathological α-syn inclusions and for the development of therapeutic strategies to optimize dopaminergic therapies for PD.

The present study we observed a similar pattern and time course of reductions in DAT binding and reductions in EDVR. EDVR is a measure of the ratio of $K_{\text{inc}}$ to $K_{\text{loss}}$ in which $k_{\text{loss}}$ reflects the rate of loss of FDOPA signal over time which is dependent on multiple factors, including DA release, DA metabolism, and DA washout. It is not clear from our study which of these processes contributed to the observed increase in DA turnover. However, our observation that reductions in EDVR mirrored reductions in DAT binding, both between surgical treatment groups and within individual PFF-injected striatum, suggests that decreased DAT contributed in decreased DA reuptake, leading to increased DA in the synaptic compartment and thus more vulnerable to metabolism and washout, increasing DA turnover. These results also mirror findings in human PD1,2 as well as similar studies performed in a 6-OHDA rat model of PD,3,5 suggesting a consistent functional role of DAT in the presence of dopaminergic deficit. Further studies will be required to directly examine the impact of PFF-seeded nigrostriatal synucleinopathy on synaptic DA levels prior to overt degeneration.

In conclusion, we observe that the nigrostriatal synucleinopathy and degenerative cascade of events triggered by intrastriatal α-syn PFF injections into rats results in
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