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α-Synuclein Expression Is Preserved in Substantia Nigra GABAergic Fibers of Young and Aged Neurotoxin-Treated Rhesus Monkeys

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Abstract

α-Synuclein (α-syn) is a small presynaptic protein distributed ubiquitously in the central and peripheral nervous system. In normal conditions, α-syn is found in soluble form, while in Parkinson's disease (PD) it may phosphorylate, aggregate, and combine with other proteins to form Lewy bodies. The purpose of this study was to evaluate, in nonhuman primates, whether α-syn expression is affected by age and neurotoxin challenge. Young adult ($n = 5$, 5–10 years old) and aged ($n = 4$, 23–25 years old) rhesus monkeys received a single unilateral carotid artery injection of 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP). Three months post-MPTP the animals were necropsied by transcardiac perfusion, and their brains extracted and processed with immunohistochemical methods. Quantification of tyrosine hydroxylase (TH)-positive substantia nigra (SN) neurons showed a significant 80–89% decrease in the side ipsilateral to MPTP administration in young and old animals. Optical density of TH- immunoreactivity (-ir) in the caudate and putamen presented a 60–70% loss compared with the contralateral side. α-Syn-ir was present in both ipsi- and contra- lateral MPTP-treated nigra, caudate, and putamen, mostly in fibers; its intracellular distribution was not affected by age. Comparison of α-syn-ir between MPTP-treated young and aged monkeys revealed significantly higher optical density for both the ipsi- and contralateral caudate and SN in the aged animals. TH and α-syn immunofluorescence confirmed the loss of nigral TH-ir dopaminergic neurons in the MPTP-treated side of intoxicated animals, but bilateral α-syn expression. Colabeling of GAD67 and α-syn immunofluorescence showed that α-syn expression was present mainly in GABAergic fibers. Our results demonstrate that, 3 months post unilateral intracarotid artery infusion of MPTP, α-syn expression in the SN is largely present in GABAergic fibers, regardless of age. Bilateral increase of α-syn expression in SN fibers of aged, compared with young rhesus monkeys, suggests that α-syn-ir may increase with age, but not after neurotoxin-induced dopaminergic nigral cell loss.

Keywords

Parkinson's disease, nonhuman primate, alpha-synuclein, tyrosine hydroxylase, age, MPTP

Introduction

α-Synuclein (α-syn) is a small presynaptic protein distributed ubiquitously in the central and peripheral nervous system. Under normal conditions, α-syn is found in soluble form, mainly in neuronal terminals. In Parkinson's disease (PD) α-syn is present as the main component of Lewy bodies (LBs)[1]. Loss of dopaminergic neurons in the substantia nigra (SN) pars compacta and the presence of LBs are the pathological hallmarks of PD. LB formation is proposed to start with abnormal accumulation and phosphorylation of α-syn in the neuronal cytoplasm[1], later forming "pale bodies", which are irregularly dense aggregates. The pale bodies then

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develop into the stereotypical dense core and translucent halo structure characteristic of LBs[2,3]. α-Syn can also abnormally accumulate in neuronal terminals forming Lewy neurites (LNs)—another typical PD pathology.

The cause of neuronal cell death, as well as the role of α-syn aggregation in PD, is still debated[4–6]. Yet, factors that increase the risk of PD onset have been identified, including increased age, exposure to environmental toxins, and genetics[7]. Current evidence links changes in α-syn to these factors. In elderly humans, α-syn has been found to accumulate in the soma of nigral dopaminergic neurons[8]. The incidence of PD diagnoses in areas of pesticide exposure further supports a role of environmental epidemiology[9,10]. Mutations in the α-syn gene, such as A30P, A53T, duplication and triplication have been associated with familial PD cases with typical nigral dopaminergic loss and presence of LBs and LNs[11–14].

Nonhuman primate (NHP) models of PD play a significant role in the advancement of how we understand and treat the disease[15]. Investigators rely on recognized risk factors to create those models; including aging, neurotoxins (e.g.: 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP)), and viral vector transfer of PD-associated genes. More recently, intranigral inoculation of α-syn fibrils or LB extracts has been attempted to shed light on the role of α-syn in PD pathogenesis[16–18]. Intraneuronal re-distribution, accumulation, phosphorylation, and aggregation of α-syn have been reported for several of these models. In intact aged monkeys, α-syn was observed to accumulate in neuronal cell bodies of the SN[8,19]. Similar observations have been found in MPTP, viral gene transfer, and LB extract models[20–28], yet identification of typical LB pathology has been elusive.

PD is an age-related disorder, yet few reports in NHP models have evaluated whether a combination of factors that include aging could trigger abnormal α-syn accumulation and LB formation[20,29–31]. In this study, we aimed to evaluate whether neurotoxin exposure at an old age would affect nigral α-syn expression, by evaluating the dopaminergic nigrostriatal system of young and old rhesus monkeys, 3 months after unilateral intracarotid artery injection of MPTP.

Materials and Methods

Ethics Statement

The present study was performed in strict accordance with the recommendations in the Guide for the Care and Use of Laboratory Animals of the National Institutes of Health (7th edition, 1996) in an AAALAC-accredited facility (Wisconsin National Primate Research Center, University of Wisconsin-Madison). The experimental protocols of the projects from which the rhesus (*Macaca mulatta*) tissues were collected were approved by the Institutional Animal Care and Use Committees at the University of Wisconsin-Madison (permit no. G00492) and at the University of Illinois-Chicago (permit no. 00-073). All efforts were made to minimize the number of animals used and to ameliorate any distress.

Animals

Coronal brain section at the level of the SN and striatum were obtained from previously published studies that utilized aged ($n = 4$, age range: 24–30 years; 7–14 kg)[32] and young ($n = 5$, age range: 5.8–10.3 years; 6–12 kg) MPTP-treated rhesus monkeys[33].

To induce parkinsonism, the monkeys received a unilateral intracarotid artery injection of MPTP-HCl (2–4 mg total dose; Sigma, St. Louis, MO, USA) in 20 ml of saline (rate: 1.33 ml/min) in sterile surgical conditions under isofluorane anesthesia as previously described. The MPTP dose was titrated according to age and weight to safely induce a similar level of parkinsonism. As MPTP is delivered via the carotid artery, and aged monkeys may increase total body weight but not brain mass, aged animals received two-thirds of the dose to be administered for a young rhesus of the same weight; e.g.: 3 mg of MPTP were given to both 7 kg young and 10 kg aged rhesus monkeys. Evaluation of the animals' parkinsonian state was done using a previously validated clinical rating scale[33]. The scale ranges from 0 to 32, with a score of 0 corresponding to normal behavior and 32 to extreme severe parkinsonian symptoms. A score of 9–13 points correspond to a stable hemiparkinsonian syndrome, and was the range that the animals in this study scored, confirming appropriate dosing.

In addition, brain sections from a transgenic mouse overexpressing human α-syn under the Thy1 promoter (courtesy of John Varghese and Asa Hatami), three young rhesus (age range: 5.2–8.1 years; 7–12 kg; from[34]) and one old rhesus monkey (age: 23.1 years; 15.64 kg; from the tissue bank at the Wisconsin National Primate Research Center, University of Wisconsin-Madison) were obtained for additional immunohistochemical controls.

MPTP-treated monkeys were euthanized 3 months post neurotoxin exposure. All animals were anesthetized with sodium pentobarbital (25 mg/kg, i.v.) and transcardially perfused with heparinized saline, followed by 4% paraformaldehyde (PFA).

Tissue Processing and Immunohistochemistry

All brains were post-fixated in 4% PFA for 12–72 h and cryoprotected by immersion in a graded (10–40%) sucrose/0.1 M phosphate-buffered saline (PBS, pH 7.2) solution. The tissue was cut frozen (40 μm section thickness) on a sliding knife microtome. All sections were stored in a cryoprotectant solution until processing.

Matching brain coronal sections of all animals were stained in parallel by immunohistochemical methods according to our previously published protocols[35–37]. Briefly, endogenous peroxidase activity was removed with a 20-min incubation in 0.1 M sodium periodate. After 3 × 10-min washes in PBS plus 0.05% Triton-X (dilution medium), background staining was blocked with a 1-h incubation in a Tris-buffered saline solution containing 3% normal donkey serum, 2% bovine serum albumin (BSA), and 0.05% Triton

X-100. Next, the sections were incubated with a primary antibody rabbit anti-α-syn (1:1000; ab138501, Abcam, Cambridge, UK), or mouse IgG1 anti-TH at 1:5000 (22941, Immunostar, Hudson, WI, USA) overnight at room temperature. Sections were then incubated for 1 h in biotinylated secondary antibodies (1:200; donkey anti-mouse BA-2000, Vector Laboratories, Burlingame, CA, USA). After 3 × 10-min washes in dilution medium, the sections were placed in the avidin biotin substrate (1:1000; ABC, "Elite" kit, Vector Laboratories) for 75 min. Afterwards, sections were washed in a 0.1 M imidazole/1.0 M acetate buffer, pH 7.4, and then reacted in a chromogen solution containing 0.05% 3,3'-diaminobenzidine (DAB), and 0.05% H_2O_2. Negative controls were performed in parallel for all immunostainings by omitting the primary antibodies during the procedures. Brain sections from a transgenic mouse overexpressing human α-syn under the Thy1 promoter were used as positive controls for α-syn-immunoreactivity (-ir). In addition, brain sections of young and old naïve rhesus monkeys were used during TH and α-syn immunohistochemistry (Suppl. Fig. 1).

Immunofluorescence

Immunofluorescence was performed in coronal brain sections to identify colocalization and distribution of α-syn with TH or GAD67 expression. After 6 × 10-min washes in PBS, nonspecific binding staining was blocked with a 2-h incubation at room temperature in a blocking buffer containing dilution medium with 5% normal serum, and 2% bovine serum albumin. The tissue was then incubated with the first primary antibody overnight at 4°C [rabbit anti-alpha-synuclein 1:1000 (ab138501, Abcam, Cambridge, UK)]. Tissues were then rinsed and incubated in PBS, secondary antibody-antigen reaction containing Alexa Fluor 594-conjugated donkey anti-rabbit antibody (1:1000; A21207, Invitrogen, Grand Island, NY, USA) for 2 h at room temperature, rinsed, and re-blocked for 2 h at room temperature, and incubated overnight at 4°C in a second primary antibody [mouse IgG2a anti-GAD67 (1:250; MAB5406, Millipore, Billerica, MA, USA) or mouse IgG1 anti-TH (1:5000; 22941, Immunostar, Hudson, WI, USA)]. Sections were then rinsed again and incubated in the secondary antibody Alexa Fluor 488-conjugated donkey anti-mouse (1:1000; A21202, Invitrogen) for 2 h at room temperature. Sections were counterstained with DAPI as a nuclei marker. Negative and positive controls were performed in parallel, as described above. Confocal images were obtained using a Nikon A1 confocal microscope (Tokyo, Japan).

Data Analysis

Optical density (OD) of caudate and putamen TH-ir and stereological TH-ir nigral cell count data were obtained from the previously published studies[32–34]. Data were normalized between studies by expressing the quantification in the MPTP-treated side as percentage of loss in relationship to the contralateral side.

Cellular localization (neuronal fibers or cell bodies) of α-syn expression was evaluated in the right and left SN (one coronal brain section per animal at the level of emergence of the 3rd cranial nerve) by three blind raters.

ImageJ software (NIH) was used to analyze OD of α-syn-ir in the SN (one coronal brain section per animal at the level of emergence of the 3rd cranial nerve), caudate, and putamen nucleus (one coronal brain section per animal at the level of the anterior commissure)[33]. To minimize immunostaining variability, matching coronal brain sections from all animals were processed in parallel (and with positive and negative controls), and were incubated in solutions for the same periods of time, using a multi-chambered dipping apparatus. Images of coronal brain sections were captured from each animal simultaneously using an Epson 1640XL-GA high-resolution digital scanner for same light exposure conditions. ImageJ was calibrated using a step tablet. OD units were obtained using the Rodbard function in order to convert grey scale values. After the optimal OD threshold was determined for each animal, a collective average threshold of 0.13 was set for OD measurements across all sections.

All data was collected and analyzed by an investigator blind to the treatment groups. Statistical analysis was done using GraphPad Prism (version 5.0b, GraphPad Software, San Diego, CA, USA). A $P < 0.05$ was accepted as significant. OD data was quantified and compared between treatment groups using one-way analysis of variance (ANOVA) and post hoc Tukey tests.

Results

Three months after intracarotid artery infusion of MPTP, young and aged rhesus monkeys displayed a unilateral pattern of TH-expression in the basal ganglia. The brain hemisphere contralateral to MPTP administration had intense TH-ir fibers in the caudate and putamen and numerous TH-ir dopaminergic neurons and fibers in the SN (Fig. 1). TH-ir cell bodies presented round or triangular morphology, with multipolar varicose neurites emanating from the perikarya. Dendrites from TH-ir somata in the SN pars compacta were observed extending ventrally to the SN pars reticulata. In contrast, the side ipsilateral to MPTP infusion presented sparse TH-ir fibers in the caudate and putamen and TH-ir nigral neurons and fibers. The few TH-ir surviving nigral neurons had smaller neuronal bodies and stunted neurites contributing to a diminished TH-ir neuropil (Fig. 1).

OD of striatal TH-ir revealed a significant 60–70% unilateral loss of dopaminergic terminals in the MPTP-treated caudate and putamen compared with the contralateral side, for both young and aged animals[32,33]. Stereological quantification of nigral TH-ir cell bodies confirmed the unilateral dopaminergic neuronal loss in the SN of young and aged MPTP-treated monkeys (Fig. 1). The percent TH-ir neuronal loss in the MPTP-treated SN compared with the unaffected

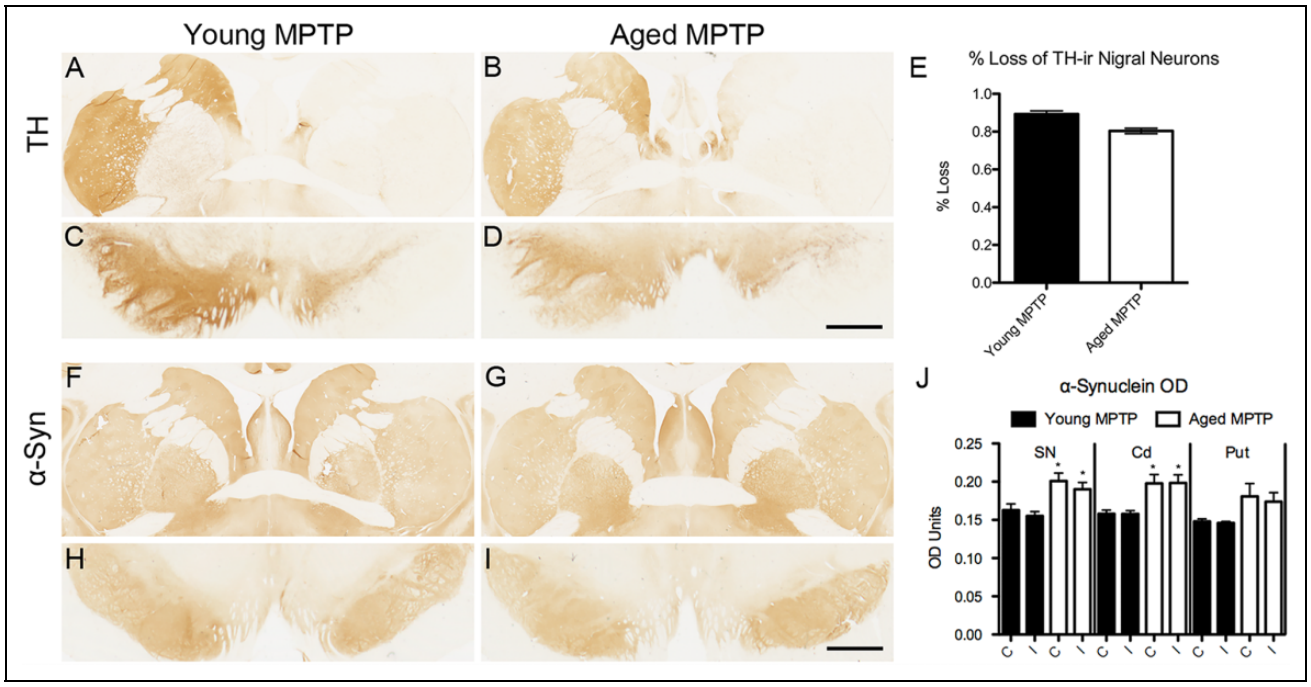

Fig 1. Evaluation of nigral dopaminergic cell loss and its effect on nigral and striatal α-synuclein optical density. Expression of tyrosine hydroxylase (TH) in the striatum (A, B) and substantia nigra (SN; C, D) of MPTP-treated young and aged rhesus monkeys. The percent loss of TH-ir cell bodies in the SN was 89.26 ± 1.71% in the young group and 80.40 ± 1.40% in aged monkeys after MPTP administration (E) and is reflected by the lack of innervation to the ipsilateral caudate and putamen. MPTP-induced loss of nigrostriatal dopaminergic innervation is similar between young and aged rhesus monkeys. Expression of α-syn in the SN, caudate and putamen was similar in the ipsilateral and contralateral MPTP-treated side (F–I). Optical density of α-syn immunolabeling was significantly increased in the SN and caudate of aged compared with young MPTP-treated monkeys; no differences were found between ipsilateral and contralateral sides to MPTP administration (J; C-contralateral, I-ipsilateral). Scale: A–D, and F–I, 2 mm.

contralateral side was 89.26 ± 1.71% in the young group and 80.40 ± 1.40% in aged monkeys.

Bilateral α-syn-ir was observed in the caudate, putamen, and SN of all monkeys, regardless of age or brain hemisphere (Fig. 1). Semi-quantitative evaluation of nigral α-syn-ir expression showed a primary presence in fibers and terminals; α-syn-ir in nigral cell somas was not observed. OD of α-syn-ir was not significantly different between ipsi vs. contralateral caudate, putamen or SN for the MPTP-treated young as well as the aged rhesus, suggesting the neurotoxin did not affect overall α-syn expression in these structures. Yet, comparison of α-syn-ir between MPTP-treated young and aged monkeys revealed significantly higher OD in the aged monkeys for both the ipsi and contralateral caudate and SN (ANOVA, post hoc Tukey's test, $P = 0.0017$ and $P = 0.0042$ respectively). In the putamen nucleus, α-syn-ir OD did not reach significance for the post hoc analysis (ANOVA $P = 0.0397$; Tukey's post hoc test test, $P > 0.05$)

To identify the neuronal population expressing α-syn in the SN after the loss of dopaminergic neurons, double-immunofluorescence staining against α-syn and TH or GAD67 was performed in coronal brain sections at the level of the mesencephalon of young and old MPTP-treated monkeys (Figs. 2 and 3). As described above, dopaminergic TH-ir cell bodies and fibers were abundant in the contralateral SN and minimal in the ipsilateral SN; α-syn-ir fibers were abundant in both SNs. Some co-localization of TH- and α-syn-ir fibers could be observed in the contralateral SN, while fibers negative for TH but positive for α-syn were clearly visible in the ipsilateral SN (Fig. 2). GAD67-ir was found bilaterally in cell bodies and fibers (Fig. 3). GAD67-positive fibers abundant in both the young and aged SN, colocalized with the preserved α-syn-ir (Fig. 3).

Discussion and Conclusions

Our results demonstrate that, 3 months after unilateral intracarotid artery infusion of MPTP, α-syn expression in the substantia nigra is present mainly in GABAergic fibers, regardless of age. The bilateral increase in α-syn expression in fibers of the nigrostriatal system of aged compared with young rhesus monkeys suggests that α-syn-ir may increase with age, but not after neurotoxin-induced dopaminergic nigral cell loss. To our knowledge, this is the first report evaluating α-syn-ir in young and aged nonhuman primates after a unilateral intracarotid artery MPTP infusion.

The presence of α-syn-ir fibers in the SN ipsilateral to MPTP was unexpected. It could be argued that MPTP

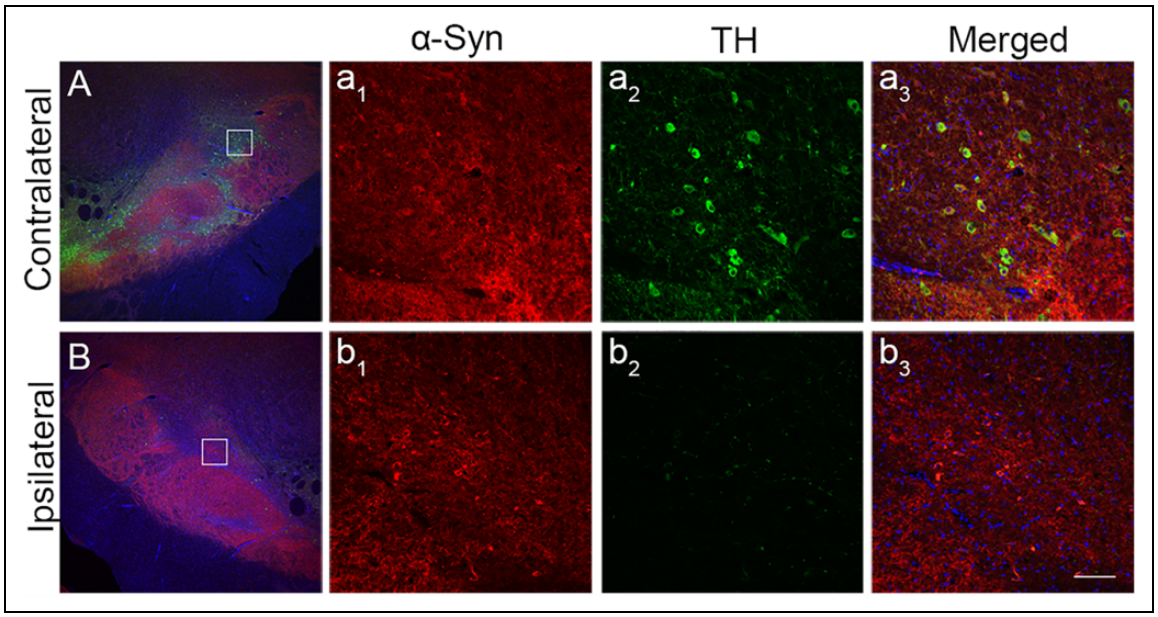

Fig 2. TH and α-syn immunofluorescence. Coronal brain images showing TH (green) and α-syn (red) immunofluorescence counterstained with DAPI (blue) in the substantia nigra contralateral (A; a1–3) and ipsilateral (B; b1–3) to a unilateral intracarotid artery infusion of MPTP in a representative rhesus monkey. TH-positive nigral neurons are lost in the ipsilateral nigra, yet abundant α-syn expression can still be observed. Scale: A and B, 500 μm; a1–3 and b1–3, 50 μm.

induced downregulation of the dopaminergic phenotype, but not neuronal death, thus α-syn-ir would be unable to colocalize with TH-ir fibers. Our group has extensive experience with the MPTP intracarotid model, and has documented, with different neuronal markers, extensive nigral dopaminergic neuronal loss 3 months post MPTP (e.g. Ohshima-Hosoyama et al. and Swanson and Emborg[33,34]). Furthermore, double immunofluorescence staining against α-syn and GAD67 confirmed that α-syn expression colocalized with GABAergic fibers. In that regard, the SN has an extensive network of GABAergic fibers and terminals emerging from striatal afferents and GABAergic nigral neurons that are impervious to catecholaminergic neurotoxins like MPTP, and, thus, preserve α-syn-ir after intoxication[38]. Therefore, evaluation of overall α-syn-ir within the nigra may not be as informative when discussing dopaminergic cell loss.

Intracellular accumulation and redistribution of nigral α-syn has been reported after MPTP administration in NHPs, yet our analysis did not identify a significant change. While we observed cell body localization of signal in the fluorescent staining, we attributed this to a technical artifact, as labeling of the cell body could not be detected in brightfield histology. A comparison with previous reports suggests that the route and dosing paradigm of MPTP administration, as well as the time lapsed after intoxication, may affect α-syn accumulation and intracellular localization. In adult baboons, postmortem brain evaluation soon after completing systemic MPTP dosing (0.4 mg/kg i.m. for 6 days and 0.27 mg/kg on the 7th day, necropsy 10 days later) found aggregation of nigral α-syn-ir in the cell body and dendrites[22]. In cynomolgus monkeys, chronic and prolonged MPTP administration (12–14 injections, 0.3 mg/kg, i.v., intermittently for 2 years beginning at age 2 and sacrificed 10 years later) was associated with accumulation and phosphorylation of α-syn in the remaining nigral neuronal cell bodies and the presence of punctate structures in the neuropil[24]. Compared with chronic systemic injections, a single MPTP administration via the intracarotid artery induces extensive (80–90%) dopaminergic neuronal death in a shorter period of time (approximately 3weeks)[39]. Analyses of α-syn expression at earlier time-points, e.g. 1 week after intracarotid MPTP, may be able to detect changes in intracellular localization and/or accumulation, during the neurodegeneration process that precedes the loss of dopaminergic nigral neurons. Otherwise, it is likely that longer experimental paradigms are necessary to observe changes in α-syn localization.

Although changes in α-syn expression have been reported after chronic MPTP administration, actual LB formation has not been confirmed in human and nonhuman primates[18]. Postmortem analysis of the brains of patients exposed to MPTP has shown poor, or no evidence of LB development. The first case report of a 23-year-old man with a survival time post MPTP of 18 months described severe nigral cell loss and one typical nigral LB observed with H&E as a "homogenous intracytoplasmic inclusion with a peripheral halo"[40]. As this publication preceded the identification of α-syn as a key element of PD pathology[3], immunohistochemistry against the protein was not performed as it was not yet available. A later report of three cases, 41–42 years old and survival times after MPTP ranging from 3 to 16 years

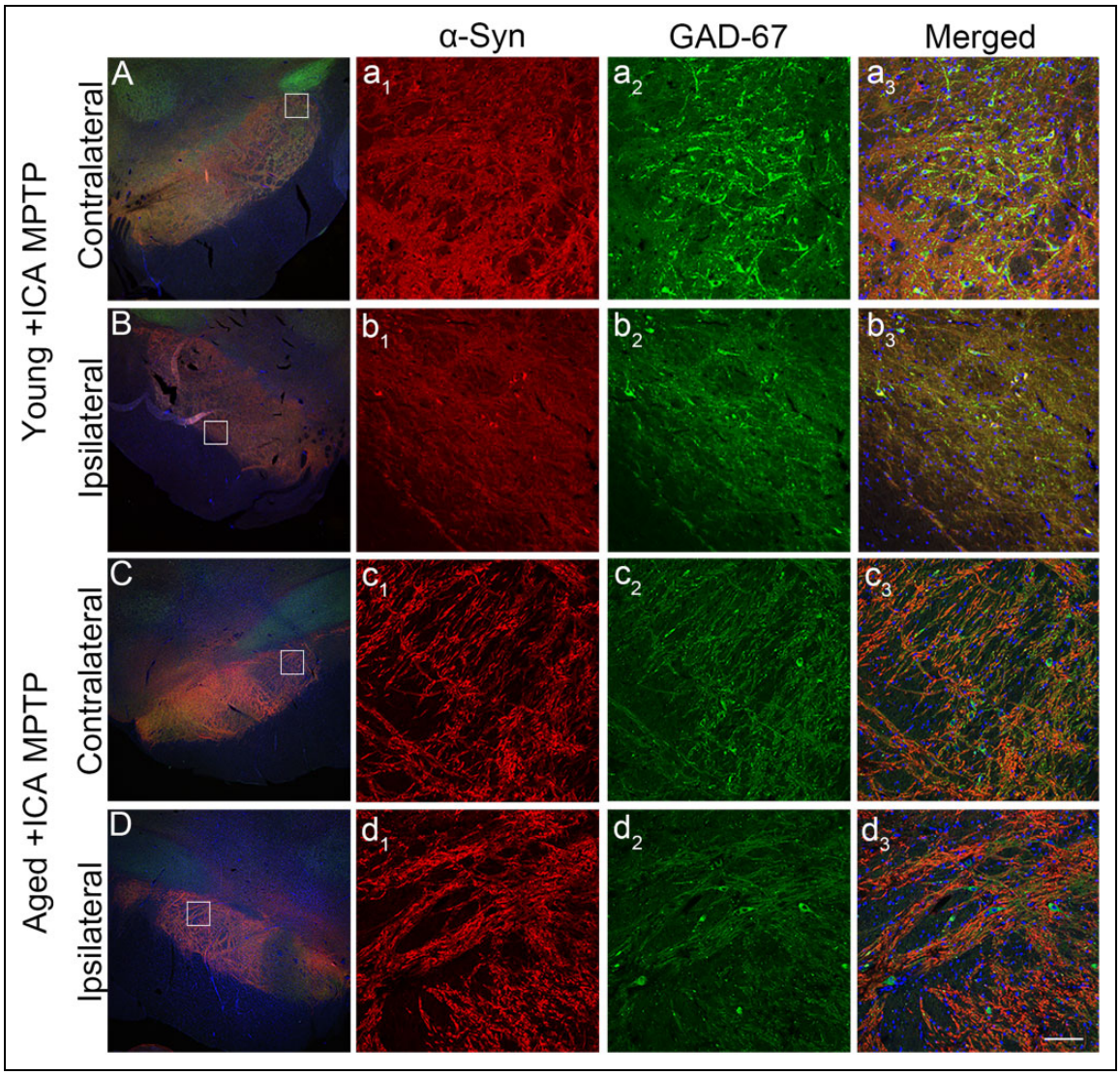

Fig 3. α-Syn and GAD67 immunofluorescence. Coronal brain images of GAD67 (green) and α-syn (red) immunofluorescence counterstained with DAPI (blue) in the substantia nigra contralateral (A, C) and ipsilateral (B, D) to a unilateral intracarotid artery (ICA) infusion of MPTP in a representative young and aged rhesus monkey. Higher magnification images (a–$d_{1\text{-}3}$) show α-syn expression in resident fibers and abundant GAD67-ir cells and fibers in the substantia nigra. Scale: A–D, 500 μm; a–$d_{1\text{-}3}$, 50 μm.

described severe nigral cell loss in the three patients, and only a few dystrophic neurites in one of the cases. Intracellular inclusions or LBs were not observed with H&E or antibodies against α-syn, ubiquitin and tau[41].

The lack of LB formation after MPTP exposure could be due to the MPTP-induced molecular mechanisms of neurodegeneration or the acute nature of the neurotoxin challenge[42]. MPTP inhibits mitochondrial complex I, which impairs ATP production and increases oxidative stress, ultimately inducing nigral cell loss. This process is further perpetuated by recruitment of immune cells and local production of inflammatory cytokines[42]. Interestingly. mitochondrial dysfunction, oxidative stress, and inflammation have all been linked to neurodegeneration in PD[4]. In this context, it seems more appropriate to consider that every MPTP dose, administered as a single or recurrent injection, induces an acute insult and rapid cell death that does not allow time for the formation of classic LBs. In that regard, controversy has surrounded the role of LBs. Some publications propose that they are toxic metabolites, while others suggest that they are a byproduct of a neuronal strategy to protect themselves by isolating harmful molecules. For either possibility, LB development has been proposed to predate the death of vulnerable neuronal populations. If MPTP-induced neurodegeneration shares mechanisms of cell death with PD, yet MPTP does not induce LBs, this strongly suggests that LB development is not driving neuronal death. With regards to our study, the accelerated and widespread nigral cell death after intracarotid artery dosing of MPTP may further decrease the period of time in which to observe changes in α-syn, especially LB formation.

Studies in squirrel monkeys have suggested that aging may facilitate α-syn accumulation and aggregation in the cell bodies after MPTP intoxication. A report from 1986 (pre-α-syn identification) describes in an aged squirrel monkey (15–20 years old) treated with MPTP (1 mg/kg i.p. twice per day for 5 days and necropsied 16 days later), the presence of eosinophilic, LB-like inclusions in the cell bodies and processes of the SN, dorsal motor nucleus of the vagus, nucleus basalis of Meynert, and dorsal raphe nucleus[29]. In a later report, old squirrel monkeys (over 12 years of age) treated with a single low dose of MPTP (1.75 mg/kg, s.c.) that induce minimal nigral cell loss (10% at 1 week and 40% at 1 month) presented nigral increases in α-syn mRNA and protein. Interestingly, α-syn-ir intraneuronal distribution changed overtime. At 1 week, α-syn-ir was evident only in neuronal fibers, while at one month 80% of surviving neurons were positive[20]. A follow up publication in animals treated with a similar MPTP paradigm and euthanized 1 month later confirmed α-syn accumulation in nigral cell bodies and dystrophic axons positive for α-syn, as well as nitrated and phosphorylated α-syn. A subset of these axons also had insoluble (proteinase K resistant α-syn aggregates), which suggests the development overtime of PD-like LB pathology[25]. Although, in our study, α-syn-ir in the nigrostriatal system was increased with age, differences between ipsilateral and contralateral MPTP treated side were not observed. As mentioned above, 3 months after intracarotid MPTP dosing, the neurodegenerative mechanisms are already completed, and extensive nigral neuronal cell loss has occurred, which may have affected the detection of changes in α-syn expression in nigral cell bodies. Due to the lack of aggregation, we did not proceed with further immunohistochemical staining.

A previous report in aged humans and naïve rhesus monkeys have shown redistribution and accumulation of soluble α-syn in nigral cell bodies[8]. It should be noted that our group reported individual variability in the intraneuronal location of α-syn-ir in the nigra of naïve adult rhesus monkeys[43]. Interestingly, in aged squirrel monkeys (over 12 years old), α-syn-ir was mainly present in axonal terminals[20]. Our current results in rhesus monkeys match the findings in squirrel monkeys, as α-syn-ir was observed mainly in neurites and terminals. Our quantification suggests that age may lead to accumulation of α-syn in fibers, with increases observed in both ipsilateral and contralateral sides to MPTP dosing in aged monkeys. Nigral OD of α-syn-ir could be affected by deposition of neuromelanin and iron, which is thought to increase with age within the nigra[44]. However, similar OD increases were found in the caudate and the putamen, suggesting α-syn accumulation with age in fibers is the likely reason for increased OD within the SN and striatum.

To conclude, a comparison of our results and other reports in young and aged MPTP-treated monkeys suggests that several factors, including age, paradigm of toxin administration, and time of evaluation after intoxication affect α-syn expression in the nigrostriatal system of nonhuman primates, and should be considered during the evaluation of the models.

Acknowledgments

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Ethical Approval

The present study was performed in strict accordance with the recommendations in the Guide for the Care and Use of Laboratory Animals of the National Institutes of Health (7th edition, 1996) in an AAALAC-accredited facility (Wisconsin National Primate Research Center, University of Wisconsin-Madison).

Statement of Human and Animal Rights

The experimental protocols of the projects from which the rhesus (*Macaca mulatta*) tissues were collected were approved by the Institutional Animal Care and Use Committees at the University of Wisconsin-Madison (permit no. G00492) and at the University of Illinois-Chicago (permit no. 00-073). All efforts were made to minimize the number of animals used and to ameliorate any distress.

Statement of Informed Consent

There are no human subjects in this article and informed consent is not applicable.

Declaration of Conflicting Interests

The author(s) declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

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Supplemental Material

Supplemental material for this article is available online.

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