Neuropsychiatric Disease and Treatment

Dovepress
Taylor & Francis Group

Open Access Full Text Article

ORIGINAL RESEARCH

Construction of a Rat Model of Hemilateral Parkinson's Disease Induced by Human Wild-Type α-Synuclein Overexpression

Qi Pan[1,*], Jianyue Lu[2,*], Zongyu Xiao[3], Huaming Zhang[4], Guanghao Liu[4], Yiying Li[5,*]

[1]Department of Neurosurgery, Sun Yat-Sen Memorial Hospital, Sun Yat-Sen University, Guangzhou, 510120, People's Republic of China; [2]International School, Sehan University, Youngam-gun, 58447, Republic of Korea; [3]Department of Neurosurgery, The Fourth Affiliated Hospital of Soochow University, Suzhou, 215124, People's Republic of China; [4]Department of Neurosurgery, the First Affiliated Hospital of Hainan Medical University, Haikou, 570102, People's Republic of China; [5]Key Laboratory of Tropical Translational Medicine of Ministry of Education & Key Laboratory of Brain Science Research Transformation in Tropical Environment of Hainan Province, School of Basic Medicine and Life Sciences, Hainan Medical University, Haikou, 571199, People's Republic of China

*These authors contributed equally to this work

Correspondence: Yiying Li, Key Laboratory of Tropical Translational Medicine of Ministry of Education & Key Laboratory of Brain Science Research Transformation in Tropical Environment of Hainan Province, School of Basic Medicine and Life Sciences, Hainan Medical University, 3# Xueyuan Road, Longhua District, Haikou, Hainan Province, 571199, People's Republic of China, Tel +8689865732303, Email liyiying_hy@163.com

Objective: Parkinson's disease (PD) is the second most prevalent neurodegenerative disorder after Alzheimer's disease. The precise etiology and pathogenesis of PD remain unclear. Human wild-type α-synuclein has been implicated in PD pathogenesis. The objective of this study is to examine the role of α-synuclein in PD by establishing a rat model of substantia nigra degeneration and Motor behavioral changes through the induced overexpression of human α-synuclein.

Methods: Rats were randomly assigned to either the Negative control group or the adeno-associated virus serotype 9 (AAV9) treatment group. Animals in the AAV9 group received 2.5 μL of AAV9 expressing human wild-type α-synuclein, while those in the Negative control group received an equal volume of AAV9 expressing green fluorescent protein via stereotactic unilateral injection into the substantia nigra pars compacta. Behavioral assessments were conducted at 1-, 3-, and 8-weeks following virus administration. Tyrosine hydroxylase and human α-synuclein expression in the substantia nigra pars compacta were analyzed. Additionally, dopamine, dihydroxyphenylacetic acid, and homovanillic acid levels in the striatum were quantified.

Results: After 3 weeks of virus induction, neurodegeneration of the right substantia nigra was observed, with a reduction in the number of tyrosine hydroxylase-immunopositive neurons in the AAV9 group. By 8 weeks, substantia nigra neurodegeneration had further progressed, and animals in the AAV9 group exhibited apomorphine-induced asymmetrical rotation and altered forelimb use.

Conclusion: Overexpression of human wild-type α-synuclein led to substantia nigra degeneration and Motor behavioral changes in rats, providing a viable model for exploring the pathogenesis of Parkinson's disease. Limitations include the 8-week observation window and the absence of neuroinflammation markers.

Keywords: α-Synuclein, dopaminergic neurons, Parkinson's disease, pathogenesis, substantia nigra

Introduction

Parkinson's disease (PD) is a prevalent progressive neurodegenerative disorder primarily affecting middle-aged and older adults. The development of robust animal models is essential for advancing basic research in this field.[1] Currently, commonly used animal models of PD are categorized into two major groups: neurotoxin-induced models and genetic models.[2–5] Neurotoxin-induced models include the 6-hydroxydopamine (6-OHDA) model, the 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine model, and the rotenone model. Genetic models encompass gene knockout and transgenic models.[6] Each of these models presents distinct advantages and limitations, yet none fully recapitulate the pathological

Received: 27 March 2025
Accepted: 22 August 2025
Published: 24 September 2025

characteristics and clinical manifestations of PD. Therefore, further research is warranted to develop a more representative and reliable animal model of PD.

While neurotoxin-based models (eg, 6-OHDA, MPTP, rotenone) reliably induce dopaminergic cell loss, they do not reproduce the α-synucleinopathy characteristic of human PD. Conversely, transgenic models often lack robust motor phenotypes or progressive neurodegeneration. These shortcomings underscore the need for a model that integrates α-synuclein pathology with behavioral and neurochemical progression.

Over the past few years, the key role of α-synuclein (α-Syn) in the pathogenesis of PD has been determined by a few studies. Mutations and gene duplication events involving α-synuclein have been identified as causative factors in familial forms of PD.[7] Additionally, single nucleotide polymorphisms (SNPs) within the α-synuclein gene have been recognized as genetic risk factors for PD, as they contribute to increased α-synuclein expression.[8] These findings indicate that elevated expression of wild-type α-synuclein may contribute to PD pathogenesis. Furthermore, in vitro studies have demonstrated that overexpression of human wild-type α-synuclein induces cellular toxicity and promotes abnormal aggregation of α-synuclein, resembling Lewy body pathology.[9]

Since the initial application of recombinant adeno-associated virus serotype 2 as a vector in genetic engineering, additional serotypes of adeno-associated virus (AAV) have been identified and isolated.[10] From these studies, it has been discovered that intravascular administration of adeno-associated virus serotype 9 (AAV9) in mice enables the virus to traverse the blood-brain barrier and transduce both bone marrow and brain tissue.[11] Furthermore, topical administration of AAV9 has been reported to be a safer and more effective approach with reduced adverse effects.[12] Neurotoxin models induce acute dopaminergic neuron death but lack Lewy body-like inclusions, whereas transgenic models often exhibit slow or incomplete degeneration and limited motor dysfunction. In contrast, AAV9-mediated α-synuclein overexpression induces progressive, region-specific degeneration with α-syn-positive inclusions, more closely mimicking human PD pathology.

Neuroinflammation is increasingly recognized as a driver of α-synucleinopathies. Misfolded α-synuclein activates microglia and astrocytes, triggering the release of IL-1β, TNF-α and other cytokines that accelerate neuronal injury and pathological spread. This bidirectional "protein-aggregate ↔ neuroinflammation" loop is therefore a critical translational target that the present model does not yet evaluate.

Recent advancements have demonstrated that a PD model based on the overexpression of the human α-synuclein gene in the rat substantia nigra using recombinant AAV represents a promising and innovative approach.[13] Kirik et al first demonstrated that AAV2-mediated overexpression of human α-synuclein in rat SNpc led to dopaminergic neuron loss and inclusion formation, but without overt motor deficits. Subsequent studies using AAV5 and AAV9 improved transduction efficiency and behavioral outcomes, showing progressive motor impairment and α-syn aggregation.[2–5] Given these findings, AAV9 was selected as the vector in this study to establish a rat model of PD through the targeted delivery of the human wild-type α-synuclein gene into the brain parenchyma.

Compared to earlier serotypes, AAV9 exhibits higher transduction efficiency, enhanced blood–brain barrier penetration, and reduced immunogenicity, making it ideal for sustained, neuron-specific expression of human wild-type α-synuclein in the rat brain. In this study, AAV9 was used as the viral vector. Stereotactic microinjection was used to administer the virus into the right substantia nigra pars compacta of rats, facilitating the overexpression of human wild-type α-synuclein and the subsequent development of a hemilateral PD model. This study aimed to evaluate whether AAV9-mediated overexpression of human wild-type α-synuclein in rat substantia nigra could recapitulate the key neuropathological and behavioral features of Parkinson's disease.

Materials and Methods

Animals

All procedures involving animals were conducted in strict accordance with ethical and welfare guidelines for laboratory animals. Approval for this study was obtained from the Experimental Animal Ethics Review Committee of Hainan Medical College.

A total of 72 healthy male Sprague-Dawley (SD) rats, weighing between 200 and 250 g, were provided by the Laboratory Animal Center of Hainan Medical College. The animals were housed in a controlled environment maintained at 22 ± 2°C, with a 12-hour light/dark cycle. A nutrient-dense diet and water were supplied ad libitum.

The rats were randomly assigned to either the Negative control (NC) group or the AAV9 treatment group. Animals in the AAV9 group received 2.5 μL of -CMV>hSNCA-WPRE vector (4.45×10^{12} GC/mL) encoding human wild-type α-synuclein, whereas those in the NC group were administered an equivalent volume of AAV9 expressing green fluorescent protein. At each time-point (1, 3 and 8 weeks), n = 12 rats per group were allocated: 6 for histopathology/immunostaining and 6 for HPLC neurochemistry (Figure 1).

For the stereotactic injection, recombinant AAV particles were delivered into the rat brain using a Stoelting (USA) stereotactic instrument. Anesthesia was induced via intraperitoneal administration of 3.5% chloral hydrate (350 mg/kg). Each rat was then secured in the stereotactic frame with the tooth bar positioned 2.4 mm lower than the ear bars. Based on the stereotaxic atlas by Paxinos and Watson (The Rat Brain in Stereotaxic Coordinates), two injection sites in the right substantia nigra pars compacta were selected. The coordinates were as follows:

First injection site: 4.8 mm posterior to the bregma, 2.1 mm lateral to the sagittal suture (right side), and 7.9 mm ventral to the dura mater.

Second injection site: 5.5 mm posterior to the bregma, 1.5 mm lateral to the sagittal suture (right side), and 7.8 mm ventral to the dura mater.

Following a midline scalp incision, a small drill was used to create an opening in the skull at the predetermined coordinates. A microsyringe was carefully inserted to the designated depth, and 2.5 μL of viral particles were injected at a rate of 0.5 μL/min. The needle was left in place for 5 minutes before being slowly withdrawn. The incision was then sutured.

Construction of AAV with Overexpression of Human SNCA Gene

HEK293T cells (CRL-11268) were purchased from ATCC (Manassas, VA, USA). HEK293T cells were cultured in cell culture flasks, and the experimental plasmid AAV-CMV>hSNCA-WPRE and the control plasmid AAV-CMV>EGFP-WPRE (purchased from Cyagen Biosciences, China) were co-transfected with the helper plasmids pHelperVector and pRCVector (AAV9) into HEK293T cells using Lipofectamine 2000 transfection reagent. Six hours after transfection, the medium was replaced with fresh complete medium. At 72 hours post-transfection, the supernatant was collected, and the

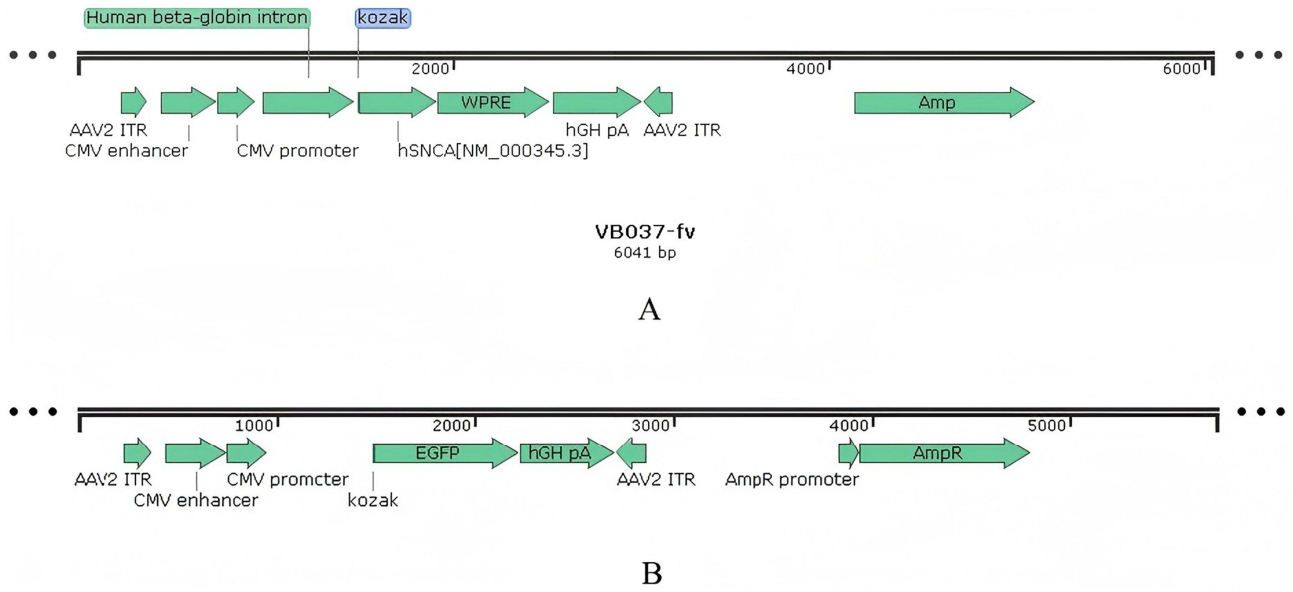

Figure 1 (**A**) Recombinant AAV vector carrying the human wild-type α-synuclein (α-Syn) gene; (**B**) Recombinant AAV vector carrying a fluorescent reporter gene.
Abbreviations: CMV, cytomegalovirus promoter; WPRE, woodchuck hepatitis post-transcriptional regulatory element; EGFP, enhanced green fluorescent protein.

https://doi.org/10.2147/NDT.S530827

remaining cells were subjected to repeated freeze-thaw cycles to release intracellular viral particles. The supernatant generated after freeze-thawing was collected by centrifugation and combined with the previously collected supernatant to generate the viral stock. Subsequently, the viral stock was purified using iodixanol density gradient centrifugation. Viral titers were quantified by qPCR and adjusted to 4.45×10^{12} GC/mL for both AAV9-CMV>hSNCA-WPRE and AAV9-CMV>EGFP-WPRE. Expression was confirmed by immunofluorescence at 3 days post-injection, with stable α-syn expression observed thereafter.

Behavioral Analysis in Rats

Voluntary Running Wheel Test

The rats in both the AAV and NC groups underwent behavioral testing using a running wheel. The running wheel had a diameter of 330 mm, a width of 80 mm, a wheel stem diameter of 6 mm, and a rod spacing of 20 mm. Signals generated during the running wheel activity were recorded and stored using magnet data acquisition software. Additionally, the running behavior of the rats was recorded using a video camera, and the captured footage was used for offline analysis of running wheel performance.

Prior to the behavioral assessment, the rats were acclimated to the running wheel for five days to ensure stabilization of their running behavior. Following this acclimation period, the rats were subjected to a 30-minute running wheel test. The primary parameters evaluated included the number of runs and the average peak velocity across multiple runs.

Asymmetry Testing for Forelimbs

The rats in each group were placed individually in a transparent plastic cylindrical chamber measuring 20 cm in diameter and 30 cm in height. Their behavior was recorded on video for a duration of 10 minutes under low-light conditions. Upon completion of the test, the recorded footage was analyzed by an evaluator blinded to the experimental groups.

The asymmetry index was calculated as described by Schallert et al[14] and Shi et al.[15] All behavioral analyses and neuronal counts were conducted by investigators blinded to group allocation. Behavioral assessment was conducted using the asymmetry index, which was calculated as follows: = (The number of times the same side touches the inner wall + half of the number of times the inner wall is contacted on both sides)/(the number of times the same side touches the inner wall + the number of times the opposite side touches the inner wall + the number of times the inner wall is contacted on both sides).

Apomorphine Induced Rotation Experiment

The rats in each group received a subcutaneous injection of apomorphine (0.25 mg/kg; Sigma, USA) and were subsequently placed in a circular test container. Observations of rotational behavior commenced 10 minutes post-injection. The number of contralateral rotations was recorded over a 30-minute period.

Staining of Substantia Nigra TH and Human α-Syn Immunofluorescence Double Label

At 1-, 3-, and 8-weeks post-virus injection, three rats from each group were randomly selected for brain tissue analysis. Following perfusion, the brains were fixed in 4% paraformaldehyde for six hours, after which paraffin sections were prepared. Tissue sections were cut at a thickness of 5 μm, followed by deparaffinization, antigen retrieval, and serum blocking. n = 6 per group per time point were used for all histological and biochemical endpoints.

Immunofluorescence staining was conducted by incubating the sections with a mouse anti- Tyrosine hydroxylase monoclonal antibody (1:100 dilution; Thermo Fisher Scientific, USA), followed by incubation with a rabbit anti-human α-synuclein (α-SYN) monoclonal antibody (1:100 dilution; Thermo Fisher Scientific, USA) and the corresponding fluorescent secondary antibody. Nuclei were counterstained using DAPI (Beyotime Biotechnology, China).

Immunohistochemical Staining and Neuronal Counting of TH in the Substantia Nigra

Tissue sections were prepared at a thickness of 5 μm. TH immunohistochemical staining was conducted following the protocol provided in the immunohistochemistry detection kit (Zhong Shan-Golden Bridge Biological, China). The mouse anti-TH primary antibody was diluted at a ratio of 1:100.

For each rat, three coronal sections were obtained at −4.8 mm, −5.2 mm, and −5.6 mm when compared to the bregma. TH-immunopositive cells in the substantia nigra pars compacta (SNc) were quantified bilaterally using a microscope (Olympus Corporation, Japan). The neuronal survival rate in the SNc was calculated as the percentage of TH-immunopositive cells on the lesioned side relative to the intact side.

Detect the Content of Dopamine, Dihydroxyphenylacetic Acid (DOPAC) and Homovanillic Acid (HVA) in the Striatum

To assess the dopamine, DOPAC, and HVA levels in the striatum following 1, 3, and 8 weeks of intervention, high-performance liquid chromatography (HPLC) was used. Three rats from each group (AAV and NC) were randomly selected at each time point. Brain tissues were extracted on ice and rapidly processed for analysis. The concentrations of dopamine, DOPAC, and HVA in the striatum were quantified by HPLC. HPLC was performed at weeks 1, 3 and 8 to capture early neurochemical changes preceding overt motor deficits. n = 6 per group per time point were used for all histological and biochemical endpoints.

Statistical Analysis

Statistical analysis was performed using SPSS 23 statistical software. Data were presented as mean ± standard deviation (SD). The spontaneous running wheel behavior test data, as well as the dopamine, DOPAC, and HVA concentrations in the striatum, were analyzed using multivariate two-way analysis of variance (ANOVA). The asymmetry index and neuronal survival rate were evaluated using univariate two-way ANOVA. The number of rotations was analyzed using the Kruskal–Wallis test. A p-value of < 0.05 was considered to indicate statistical significance.

Results

Behavioral Test results

Voluntary Wheel Running Test

To assess behavioral changes following viral injection, a voluntary wheel running test was conducted. As depicted in Figure 2, during the first week post-injection, both the NC and AAV groups exhibited similar numbers of runs and average peak velocity, with no statistically significant differences between the groups.

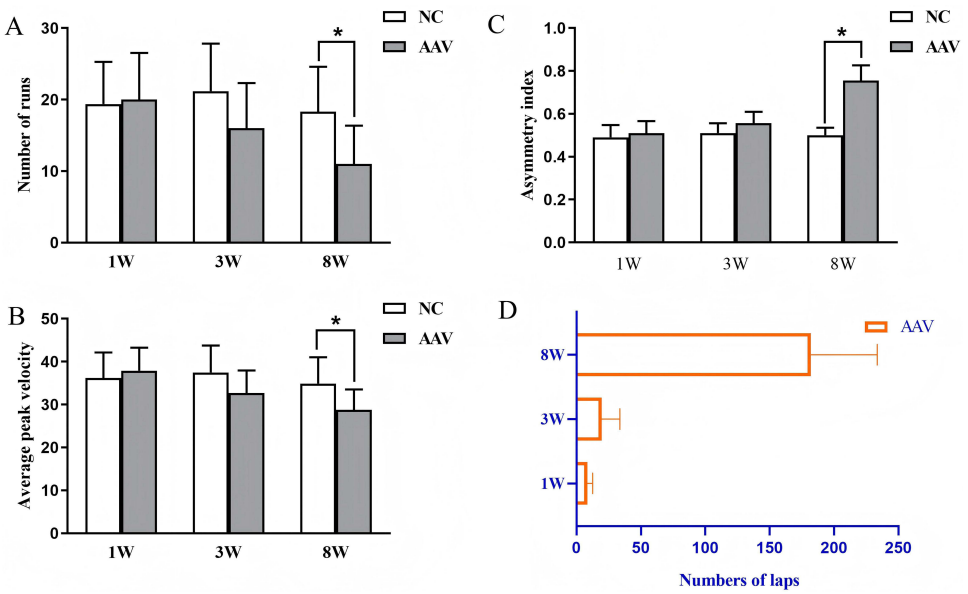

Figure 2 Voluntary running wheel test results at 1-, 3-, and 8-weeks post-virus injection. (**A**) Number of runs; (**B**) Average peak velocity. (**C**) Forelimb asymmetry test results at 1-, 3-, and 8-weeks post-virus injection. (**D**) Number of contralateral rotations in the AAV group at 1-, 3-, and 8-weeks post-virus injection, recorded over 30 minutes. *$p < 0.05$.

Abbreviations: NC, Negative control group; AAV, adeno-associated virus serotype 9 group.

https://doi.org/10.2147/NDT.S530827

At three weeks post-injection, a partial reduction in the number of runs and average peak velocity was observed in the AAV group; however, the difference remained statistically non-significant compared with the NC group. By eight weeks post-injection, a significant decline in both the number of runs and the average peak velocity was observed in the AAV group, with the difference reaching statistical significance compared with the NC group.

Asymmetry Test for Forelimbs

To evaluate behavioral changes in rats following viral injection, an asymmetry test for forelimb use was conducted. As depicted in Figure 2C, at one-week post-injection, the asymmetry indices for the NC and AAV groups were 0.49 and 0.51, respectively, with no statistically significant difference between the groups ($F = 0.644$, $p = 0.426$).

At three weeks post-injection, the asymmetry indices for the NC and AAV groups were 0.51 and 0.56, respectively, and the difference remained statistically non-significant ($F = 3.602$, $p = 0.063$).

By eight weeks post-injection, the asymmetry indices for the NC and AAV groups were 0.50 and 0.76, respectively, with a statistically significant difference between the groups ($F = 111.826$, $p < 0.001$). The asymmetry index in the AAV group exceeded 0.5, indicating a preferential use of the limb on the injected side. These data indicate early motor asymmetry in the AAV group.

Apomorphine-Induced Rotation Experiment

At 8 weeks post-virus injection, rats in the experimental group exhibited continuous contralateral rotation following apomorphine (APO) administration, characterized by rapid rotation with the unaffected hind limb serving as the pivot, while the head and tail remained aligned and the body curved into a circular posture. The number of rotations within 30 minutes at 1-, 3-, and 8-weeks post-injection was 7.9±4.5, 18.5±13.6, and 188.1±50.5, respectively (Figure 2D). Statistical analysis revealed significant differences across these time points ($H=21.123$, $P<0.001$). Specifically, the number of rotations at 8 weeks was significantly higher than at 1 and 3 weeks ($P<0.001$; $P=0.005$), while no significant difference was observed between 1 and 3 weeks ($P=0.559$). Apomorphine failed to induce rotational behavior in the control group rats. At 8 weeks, a significant reduction in both the number of runs and peak velocity was observed in the AAV group ($\eta^2 = 0.52$, 95% CI: 0.31–0.71).

Immunofluorescence Double-Labeled Staining

To assess the expression of TH immunopositive neurons and human α-synuclein in rats, immunofluorescence double-label staining was conducted. As depicted in Figure 3, at one-week post-virus injection, no detectable expression of human wild-type α-synuclein was observed in the substantia nigra on the injected side in the AAV group. No significant difference in striatal dopamine levels was observed at week 1. Similarly, no green fluorescent protein (GFP) expression was detected in the substantia nigra of the NC group. In both groups, abundant TH-immunopositive neurons were present in the substantia nigra on the injected side.

At three weeks post-injection, human wild-type α-synuclein expression became evident in the substantia nigra of the AAV group, while GFP expression was observed in the substantia nigra of the NC group. Compared to the NC group, the density of TH-immunopositive neurons in the substantia nigra of the AAV group appeared slightly reduced.

By eight weeks post-injection, a marked reduction in TH-immunopositive neurons was observed in the substantia nigra of the AAV group. The 8-week decrease in TH-positive neurons showed a large effect size ($\eta^2 = 0.68$, 95% CI: 0.55–0.78). Additionally, multiple α-synuclein-positive inclusions were detected (Figure 3, yellow arrows). In contrast, the substantia nigra tissue on the injected side of the NC group revealed no significant changes in expression.

Immunohistochemical Staining and Neuronal Counting

To evaluate the expression of TH-immunopositive neurons in the substantia nigra, immunohistochemistry and neuronal counts were conducted. As depicted in Figure 4, at one-week post-virus injection, a large number of TH-immunopositive neurons were present in the substantia nigra on both sides in both the NC and AAV groups.

At three- and eight-weeks post-injection, a significant reduction in the survival rate of TH-immunopositive neurons was observed in the AAV group compared to the NC group ($F = 98.455$, $p < 0.001$; Figure 4). Neuronal count analysis

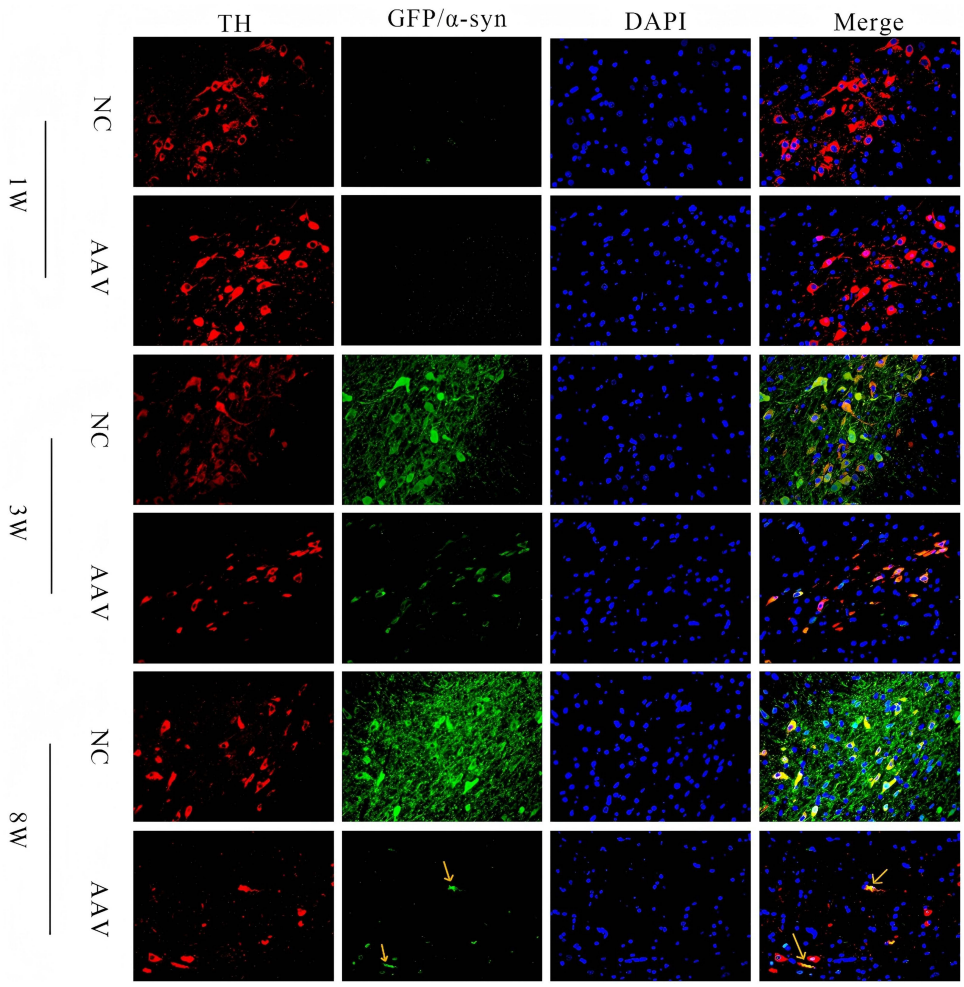

Figure 3 Expression of TH-immunopositive neurons and α-synuclein in the substantia nigra of rats at 1-, 3-, and 8-weeks post-virus injection. Yellow arrows indicate α-synuclein-positive inclusion bodies. Magnification: 400×.
Abbreviations: NC, Negative control group; AAV, adeno-associated virus serotype 9 group; TH, TH-immunopositive; GFP, green fluorescent protein; DAPI, 4',6-diamidino-2-phenylindole. Scale bars = 100 μm.

revealed that, at three and eight weeks post-injection, the number of TH-immunopositive neurons in the SNc on the lesioned side of the AAV group decreased by 24.7% and 78.2%, respectively, compared to the NC group.

Dopamine, DOPAC, and HVA Content

To assess the dopamine, DOPAC, and HVA concentrations in the striatum following 1, 3, and 8 weeks of virus injection, HPLC was conducted. As depicted in Figure 5, at one-week post-injection, no significant changes were observed in the dopamine, DOPAC, and HVA levels in the bilateral striatum of rats in either the AAV or NC groups.

However, at two- and three-weeks post-injection, the levels of dopamine, DOPAC, and HVA in the right striatum of rats in the AAV group were significantly reduced compared to the NC group. Additionally, in the AAV group, the concentrations of dopamine, DOPAC, and HVA in the right striatum were significantly lower than those in the left hemisphere, indicating asymmetric neurochemical changes.

Discussion

In this study, stereotactic microinjection technology was used to deliver a recombinant AAV9 vector overexpressing human wild-type α-synuclein into the SNc on one side of the rat brain, thereby establishing a hemilateral PD model based on α-synuclein overexpression.

https://doi.org/10.2147/NDT.S530827

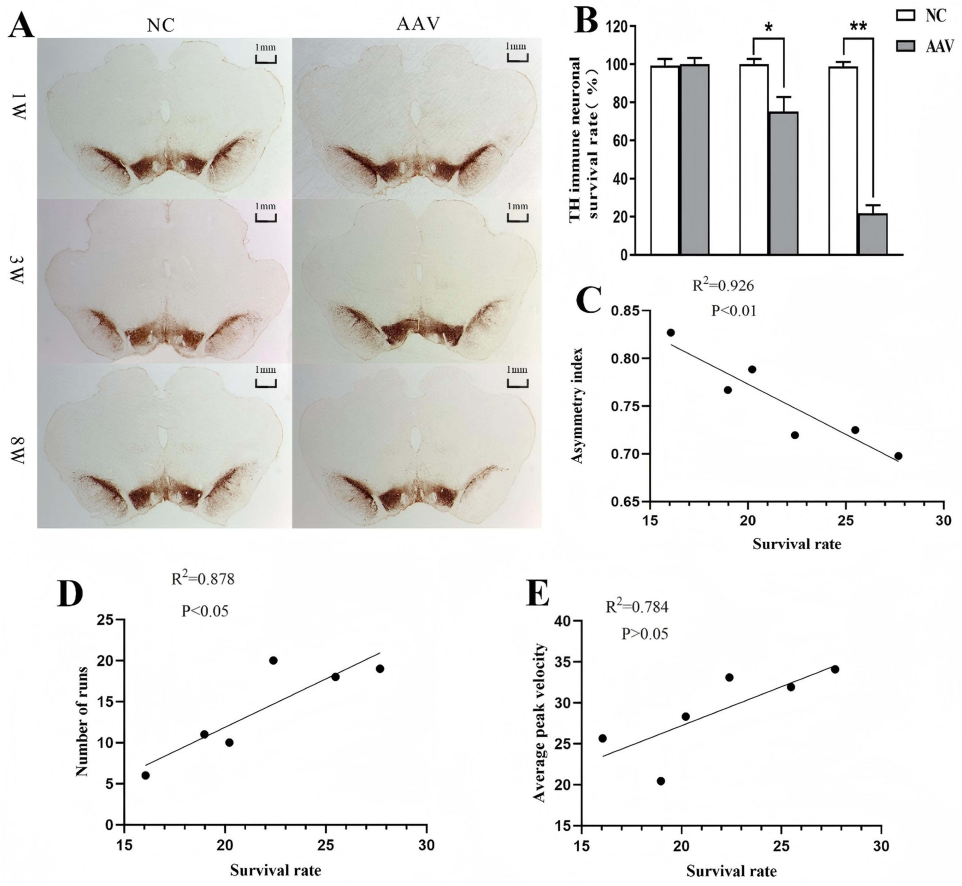

Figure 4 Immunohistochemical staining and neuronal quantification of TH-immunopositive neurons in the substantia nigra at 1-, 3-, and 8-weeks post-virus injection. (**A**) Immunohistochemical staining; (**B**) Neuronal count; (**C**) Correlation between neuronal survival rate and asymmetry index in the AAV group at 8 weeks post-injection; (**D**) Correlation between neuronal survival rate and number of runs in the AAV group; (**E**) Correlation between neuronal survival rate and average peak velocity in the AAV group. *, ** denote $p < 0.05$ and $p < 0.01$ vs NC group, respectively; Magnification: 400×.
Abbreviations: NC, Negative control group; AAV, adeno-associated virus serotype 9 group.

Parkinson's disease is a progressive neurodegenerative disorder and one of the leading causes of neurological disability. It is clinically characterized by tremor and bradykinesia, though the precise etiology remains undetermined. Increasing evidence indicates that α-synuclein plays a key role in the pathogenesis of PD.[16,17]

α-Synuclein is a 140-amino acid protein primarily localized in the presynaptic terminals of neurons. It has been functionally implicated in SNARE complex formation, thereby regulating vesicle trafficking and neurotransmitter release. Additionally, α-synuclein is the primary component of Lewy bodies, a hallmark pathological feature of PD. Mutations and gene duplication events affecting α-synuclein have been identified as causative factors in familial PD.[18] The neurotoxic effects of excess α-synuclein are multifaceted and involve nuclear dysfunction, mitochondrial impairment, lysosomal dysfunction, disruption of calcium ion homeostasis, and synaptic abnormalities.[19,20] Furthermore, SNPs within the α-synuclein gene have been identified as genetic risk factors for PD, with certain SNPs contributing to increased α-synuclein expression.[21]

In this study, recombinant AAV9 encoding human wild-type α-synuclein was injected into the substantia nigra pars compacta on one side of the rat brain. The results demonstrated that dopaminergic neurons in the injected substantia nigra exhibited high levels of human wild-type α-synuclein expression. By eight weeks post-injection, a significant reduction in dopaminergic neurons was observed in the substantia nigra of the injected hemisphere, accompanied by the presence of multiple α-synuclein-positive inclusions. These findings are consistent with those reported by Kirik et al.[22] Collectively, the results indicate that overexpression of wild-type α-synuclein contributes to neurodegeneration, supporting its role in the pathogenesis of Parkinson's disease, as depicted in Figure 6. Although a CMV promoter was used, the

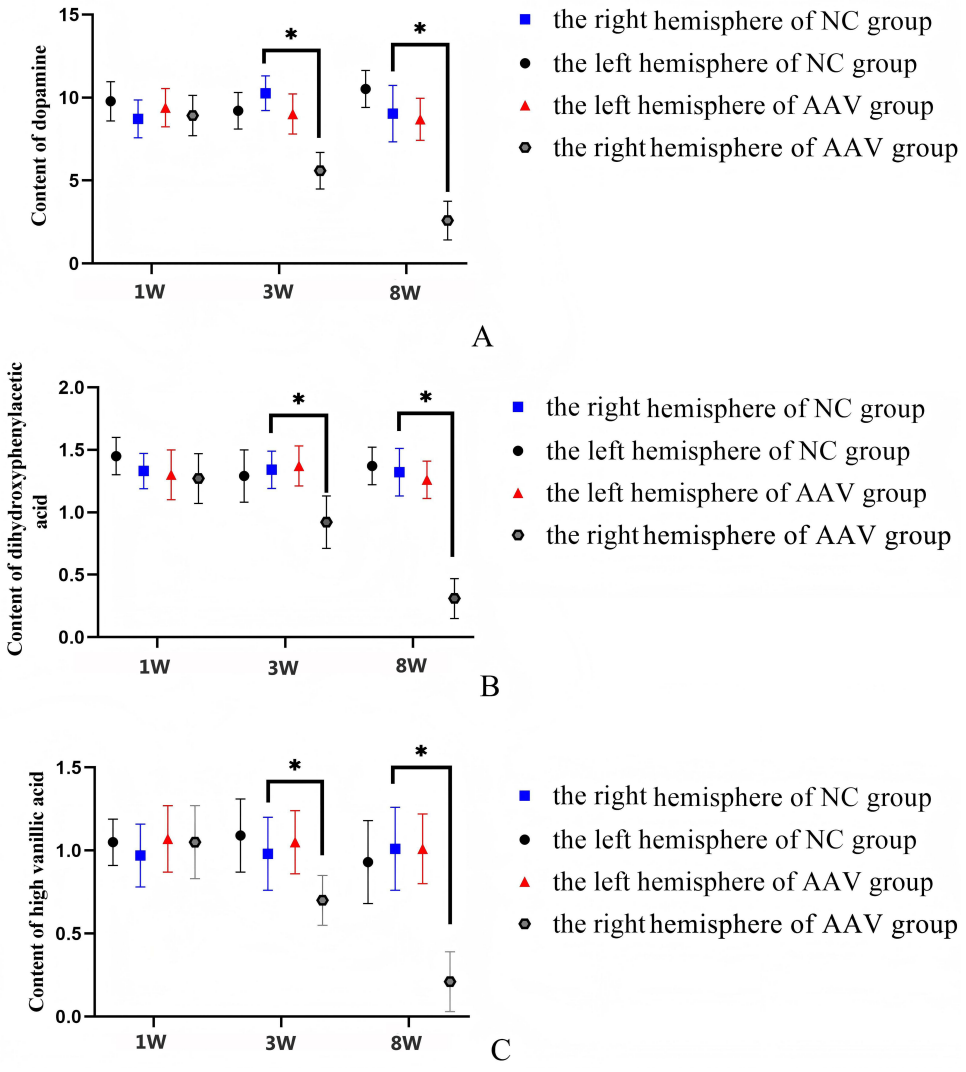

Figure 5 Concentration of dopamine, dihydroxyphenylacetic acid, and homovanillic acid in the striatum. Dopamine levels in the right (injected side) striatum (ng/mg tissue). n = 6 per group per time point. (**A**) Dopamine levels; (**B**) DOPAC levels; (**C**) HVA levels. *$p < 0.05$.
Abbreviations: NC, Negative control group; AAV, adeno-associated virus serotype 9 group.

observed restriction of α-syn expression to TH-positive neurons is likely due to preferential transduction of dopaminergic cells by AAV9 and post-transcriptional regulation within these neurons. Compared with classic 6-OHDA, MPTP, or transgenic models, the AAV9-α-syn model reproduces α-synucleinopathy, progressive dopaminergic loss, and behavioral deficits, while avoiding acute toxicity or developmental compensation seen in germline transgenics.

The animal model established in this study exhibited significant motor impairment, as assessed through commonly used motor behavior tests. The voluntary running wheel task is a widely used method for evaluating motor function, and various experimental animals, including rodents, demonstrate a strong preference for this activity.[23] Voluntary running behavior is considered a spontaneous locomotor activity that is highly adaptable and influenced by multiple intrinsic and extrinsic factors. Prior studies demonstrated impaired voluntary running behavior in rats with 6-OHDA-induced hemiparkinsonism, further supporting the validity of this method in evaluating PD models.[24] These findings indicate that spontaneous running behavior assessments could be used as a screening tool for anti-PD therapeutics and other interventions.

In this study, two key parameters were used in the voluntary wheel running test: the number of runs and the average peak velocity. The number of runs and the peak velocity of each running bout serve as indicators of motor capacity and limb function in rats. The results demonstrated a significant reduction in both parameters in the AAV group, indicating

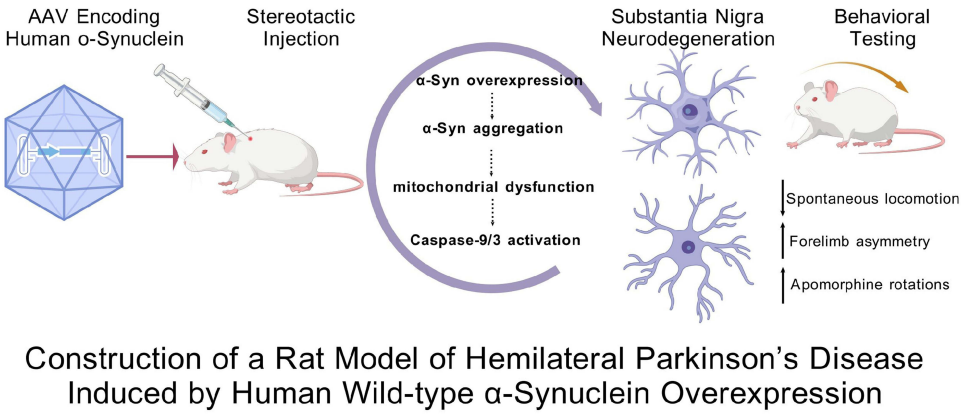

Figure 6 Schematic representation of a hemilateral PD rat model, induced by the intrastriatal injection of human wild-type α-synuclein.
Abbreviation: AAV9, adeno-associated virus serotype 9.

motor deficits. A decrease in running frequency indicates a decline in overall locomotor activity, reflecting motor impairment in the PD model rats.

Additionally, correlation analysis was conducted between neuronal survival rate and asymmetry index, number of laps, and average peak velocity. As depicted in Figure 4, a significant correlation was observed between neuronal survival rate and asymmetry index, as well as between neuronal survival rate and number of laps. However, no significant correlation was found between neuronal survival rate and average peak velocity.

Furthermore, neuronal quantification revealed that at eight weeks post-virus injection, the number of TH-immunopositive neurons in the injured SNc of the AAV group had decreased by 78.2%, a finding corroborated by immunohistochemistry and immunofluorescence analyses. Additionally, a significant reduction in the dopamine, DOPAC, and HVA content in the right striatum of AAV group rats provided indirect confirmation of neuronal damage in the PD model.

Limitations include the 8-week follow-up, which may not capture the full chronicity of human PD, lack of Braak staging or cortical spread was not evaluated and the absence of microglial (Iba-1) or astrocytic (GFAP) markers. Compared with pre-formed fibril (PFF) or A53T transgenic models, the AAV9-wt-α-syn approach yields more rapid transduction but lacks the temporal fidelity of germline models. Future work will incorporate longitudinal PET neuroinflammation imaging and late-stage behavioral assessments.

Conclusion

In conclusion, the findings of this study demonstrate that rats injected with wild-type α-synuclein exhibit a significant reduction in substantia nigra neurons and develop motor impairments characteristic of PD. The progression of neuropathological changes occurs gradually over time, with the presence of α-synuclein-positive inclusions and dystrophic neurites, closely resembling the pathological features observed in idiopathic PD. While this model recapitulates key motor and neuropathological features, its short follow-up and lack of neuroinflammation data should be considered when interpreting therapeutic outcomes.

These results indicate that this AAV9-α-syn model recapitulates key features of PD—including progressive nigrostriatal degeneration, α-syn aggregation, and motor deficits—while acknowledging limitations such as lack of cortical pathology. It is well-suited for evaluating disease-modifying therapies, including α-syn aggregation inhibitors, anti-inflammatory agents, and neuroprotective strategies.

Abbreviations

AAV9, adeno-associated virus 9; PD, Parkinson's disease; NC, Negative control; 6-OHDA, 6-Hydroxydopamine; MPTP, 1-methyl-4-phenyl-1,2,3,6-te-trahydropyridine; SD, Sprague Dawley; DAPI, 4',6-diamidino-2-phenylindole; TH, Tyrosine hydroxylase; SNc, compact part of substantia nigra; HPLC, high-performance liquid chromatography.

Data Sharing Statement

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

Ethics Approval

All animal experiments were approved by the Institutional Animal Care and Use Committee at the Hainan Medical University (HYLL-2021-119). All procedures followed the ARRIVE 2.0 guidelines and the Guide for the Care and Use of Laboratory Animals (NIH Publication No. 8023, revised 2011).

Funding

This work was supported by Hainan Provincial Natural Science Foundation of China (821RC698), Hainan Provincial Natural Science Foundation of China (821QN260) and the Project of Hainan Association for Science and Technology of Youth Science and Talents (QCXM202023).

Disclosure

None of the authors have any financial disclosure or conflict of interest for this work.

References

1. Jagadeesan AJ, Murugesan R, Vimala Devi S, et al. Current trends in etiology, prognosis and therapeutic aspects of Parkinson's disease: a review. *Acta Biomed*. 2017;88(3):249–262. doi:10.23750/abm.v88i3.6063
2. Dawson TM, Ko HS, Dawson VL. Genetic animal models of parkinson's disease. *Neuron*. 2010;66(5):646–661. doi:10.1016/j.neuron.2010.04.034
3. Berg J, Roch M, Altschüler J, et al. Human adipose-derived mesenchymal stem cells improve motor functions and are neuroprotective in the 6-hydroxydopamine-rat model for Parkinson's disease when cultured in monolayer cultures but suppress hippocampal neurogenesis and hippocampal memory function when cultured in spheroids. *Stem Cell Rev Rep*. 2015;11(1):133–149. doi:10.1007/s12015-014-9551-y
4. Guo M. Drosophila as a model to study mitochondrial dysfunction in Parkinson's disease. *Cold Spring Harb Perspect Med*. 2012;2(11):a009944. doi:10.1101/cshperspect.a009944
5. Matsui H, Uemura N, Yamakado H, Takeda S, Takahashi R. Exploring the pathogenetic mechanisms underlying Parkinson's disease inmedaka fish. *J Parkinsons Dis*. 2014;4(2):301–310. doi:10.3233/JPD-130289
6. Schmidt WJ, Alam M. Controversies on new animal models of Parkinson's disease pro and con: the rotenone model of Parkinson's disease (PD). *J Neural Transm Suppl*. 2006;(70):273–276.
7. Mizuno Y, Hattori N, Mori H. Genetics of Parkinson's disease. *Biomed Pharmacother*. 1999;53(3):109–116. doi:10.1016/S0753-3322(99)80075-4
8. Diaz-Ortiz ME, Seo Y, Posavi M, et al. GPNMB confers risk for Parkinson's disease through interaction with α-synuclein. *Science*. 2022;377 (6608):eabk0637. doi:10.1126/science.abk0637
9. Vasili E, Dominguez-Meijide A, Flores-León M, et al. endogenous levels of alpha-synuclein modulate seeding and aggregation in cultured cells. *Mol Neurobiol*. 2022;59(2):1273–1284. doi:10.1007/s12035-021-02713-2
10. Hermonat PL, Muzyczka N. Use of adeno-associated virus as a mammalian DNA cloning vector: transduction of neomycin resistance into mammalian tissue culture cells. *Proc Natl Acad Sci USA*. 1984;81(20):6466–6470. doi:10.1073/pnas.81.20.6466
11. Foust KD, Nurre E, Montgomery CL, Hernandez A, Chan CM, Kaspar BK. Intravascular AAV9 preferentially targets neonatal neurons and adult astrocytes. *Nat Biotechnol*. 2009;27(1):59–65. doi:10.1038/nbt.1515
12. Merentie M, Lottonen-Raikaslehto L, Parviainen V, et al. Efficacy and safety of myocardial gene transfer of adenovirus, adeno-associated virus and lentivirus vectors in the mouse heart. *Gene Ther*. 2016;23(3):296–305. doi:10.1038/gt.2015.114
13. Pingale T, Gupta GL. Classic and evolving animal models in Parkinson's disease. *Pharmacol Biochem Behav*. 2020;199:173060.
14. Schallert T. Behavioral tests for preclinical intervention assessment. *NeuroRx*. 2006;3(4):497–504. doi:10.1016/j.nurx.2006.08.001
15. Shi X, Xing F, Xu K, Sapkota M, Yang L. Asymmetric discrete graph hashing. *Procee AAAI Conf Artif Intellig*. 2017;31(1). doi:10.1609/aaai.v31i1.10831
16. Karikari AA, McFleder RL, Ribechini E, et al. Neurodegeneration by α-synuclein-specific T cells in AAV-A53T-α-synuclein Parkinson's disease mice. *Brain Behav Immun*. 2022;101:194–210. doi:10.1016/j.bbi.2022.01.007
17. Badr M, McFleder RL, Wu J, et al. Expansion of regulatory T cells by CD28 superagonistic antibodies attenuates neurodegeneration in A53T-α-synuclein Parkinson's disease mice. *J Neuroinflammation*. 2022;19(1):319. doi:10.1186/s12974-022-02685-7
18. Mehra S, Sahay S, Maji SK. α-Synuclein misfolding and aggregation: implications in Parkinson's disease pathogenesis. *Biochim Biophys Acta Proteins Proteom*. 2019;1867(10):890–908. doi:10.1016/j.bbapap.2019.03.001
19. Ma K-L, Song L-K, Yuan Y-H, et al. The nuclear accumulation of alpha-synuclein is mediated by importin alpha and promotes neurotoxicity by accelerating the cell cycle. *Neuropharmacology*. 2014;82:132–142. doi:10.1016/j.neuropharm.2013.07.035
20. Pan L, Li C, Meng L, et al. Tau accelerates α-synuclein aggregation and spreading in Parkinson's disease. *Brain*. 2022;145(10):3454–3471. doi:10.1093/brain/awac171
21. Prahl J, Coetzee GA. Genetic elements at the alpha-synuclein locus. *Front Neurosci*. 2022;16:889802. doi:10.3389/fnins.2022.889802

22. Kirik D, Annett LE, Burger C, Muzyczka N, Mandel RJ, Björklund A. Nigrostriatal α-synucleinopathy induced by viral vector-mediated overexpression of human α-synuclein: a new primate model of Parkinson's disease. *Proc Natl Acad Sci USA*. 2003;100(5):2884–2889. doi:10.1073/pnas.0536383100
23. Willuhn I, Steiner H. Motor-skill learning in a novel running-wheel task is dependent on D1 dopamine receptors in the striatum. *Neuroscience*. 2008;153(1):249–258. doi:10.1016/j.neuroscience.2008.01.041
24. Pan Q, Zhang W, Wang J, Luo F, Chang J, Xu R. impaired voluntary wheel running behavior in the unilateral 6-hydroxydopamine rat model of parkinson's disease. *J Korean Neurosurg Soc*. 2015;57(2):82–87. doi:10.3340/jkns.2015.57.2.82