Received: 27 April 2022 | Revised: 13 August 2022 | Accepted: 15 August 2022

DOI: 10.1002/syn.22249

RESEARCH ARTICLE

SYNAPSE WILEY

Acute MPTP treatment decreases dendritic spine density of striatal medium spiny neurons via SNK-SPAR pathway in C57BL/6 mice

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Funding information
Basic scientific research project of colleges and universities of Liaoning Provincial Department of Education, Grant/Award Number: LJKZ1147

Abstract

Parkinson's disease (PD) is a well-known neurodegenerative disorder associated with a high risk in middle-aged and elderly individuals, severely impacting the patient's quality of life. 1-Methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) is frequently used to establish PD in animals. Dendritic spines are dendritic processes that form the foundation of learning and memory. Reportedly, dendritic spine density of striatal medium spiny neurons (MSNs) declines in PD, and this decline has been associated with PD progression; however, the underlying mechanism remains elusive. Herein, we used the MPTP animal model to examine whether serum-induced kinase (SNK) and spine-associated Rap guanosine triphosphatase (SPAR) contribute to decreased dendritic spine density in striatal MSNs. MPTP was used to establish the animal model, which exhibits motor function impairment and dopaminergic cell loss. To assess spine density, Golgi staining was performed to count striatal dendritic spines, which were reduced in the MPTP group when compared with those in the normal control group. Immunohistochemistry was performed to analyze changes in SNK and SPAR expression. MPTP treatment significantly increased the expression of SNK in striatal MSNs, whereas that of SPAR was significantly decreased when compared with the normal control group. These findings offer clues to further explore the mechanism of declining dendritic spine density in patients with PD and provide evidence for potential target identification in PD.

KEYWORDS
MPTP, PD, dendritic spine density, SNK, SPAR, MSNs

1 | INTRODUCTION

Parkinson's disease (PD) is a common neurodegenerative disorder characterized by the pathological hallmark of dopaminergic (DA) cell loss in the substantia nigra. Most patients experience rigidity, tremor, and bradykinesia, described as Parkinsonism (Bloem et al., 2021; Kumar, 2018). It is well-established that 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP), a neurotoxin, can induce Parkinsonism-like symptoms, and MPTP is typically employed to establish experimental models of PD in animals (Jackson-Lewis & Przedborski, 2007; Jenner, 2003). Following MPTP treatment, DA cell loss and reduced tyrosine hydroxylase (TH) can be observed in the substantia nigra, as determined using immunohistochemistry (IHC) (Furune et al., 1989). Furthermore, MPTP reportedly reduces the dendritic spine density of medium spiny neurons (MSNs) in the striatum

(Villalba et al., 2009). Dendritic spine morphology is reportedly regulated by serum-inducible kinase (SNK) and spine-associated Rap guanosine triphosphatase activating protein (SPAR), coupled with postsynaptic density (PSD)-95 (Pak & Sheng, 2003; Pak et al., 2001).

Striatal MSNs receive converging DA and glutamatergic inputs and process most afferent information (Arbuthnott & Wickens, 2007). In MPTP-induced mice, remodeling dendritic spines of striatal MSNs was found to enhance Parkinsonism-like symptoms (Antzoulatos et al., 2011). Furthermore, striatal MSNs were shown to be involved in cocaine addiction; hence, establishing the mechanism of dendritic spine remodeling can have marked implications (Villalba & Smith, 2013). Although dendritic spine remodeling plays a critical role in neurodegenerative diseases, its underlying mechanism remains unknown (Herms & Dorostkar, 2016). Considering the MPTP-lesion model, available reports have primarily focused on screening therapeutic agents to alleviate disease, especially in the hippocampus (Shin et al., 2016; Wang et al., 2019). The SNK-SPAR pathway affords a potential mechanism to clarify the remodeling of dendritic spines. In vitro, the SNK-SPAR pathway was shown to participate in glutamate excite-neurotoxin in primary mouse hippocampus cell culture, previously unconfirmed in histological examination (Wu et al., 2007). Beta-amyloid is the hallmark of Alzheimer's disease and promotes loss of dendritic spines in hippocampal neurons of mice via the SNK-SPAR pathway (Gong et al., 2010). Microwave radiation reportedly induces memory disorder owing to lesions in the dendritic spine ultrastructure via the SNK-SPAR pathway (Zhi et al., 2018).

Most previous research has focused on the hippocampal region, with minimal data from other brain regions regarding altered dendritic spine morphology associated with the SNK-SPAR pathway. In addition, the reduction in dendritic spine density in the MPTP animal model needs to be comprehensively established. Herein, we examined the relationship between altered dendritic spine density in striatal MSNs and the SNK-SPAR pathway following MPTP treatment. Immunohistochemistry (IHC) was employed to examine the expression of SNK and SPAR. We confirmed the expression of SNK and SPAR in histological assessments, accompanied by altered dendritic spine density of MSNs in the striatum of the MPTP-lesion animal model.

2 | MATERIALS AND METHODS

2.1 | Animal experiments

Male C57BL/6 mice ($n = 8$; 8-week-old and ~20 g in weight) were obtained from Liaoning Changsheng Biotechnology co., Ltd. (Liaoning, China) and housed in cages (22–24°C and 40%–60% humidity) under a 12-h light/dark cycle. Ethics approval was obtained from the Ethics Committee of Shenyang Medical College. In addition, Shenyang Medical College approved the experimental protocol. This study was performed in accordance with the National Institutes of Health Laboratory Animal Care and Use Guidelines. Mice were randomly divided into two groups: normal control (NC, $n = 4$) and MPTP treatment (MPTP, $n = 4$). MPTP (25 mg/kg; GlpBio, Shanghai, China), dissolved in saline, was administered intraperitoneally at four-time intervals for 2 h; the NC group was administered 25 mg/kg saline intraperitoneally at four-time intervals for 2 h (Jackson-Lewis & Przedborski, 2007). After the last injection, behaviors were tested at intervals of 1, 3, and 8 days, and animals were subsequently sacrificed. Brains were collected for Golgi staining and immunohistochemistry (IHC). One hemisphere was fixed with 4% paraformaldehyde; the other was prepared according to Golgi-Cox OptimStain™ Kit (Hitobiotec, Kingsport, USA) instructions.

2.2 | Swim test

As described by Donnan et al. (1987), mice were placed in a plastic box (20 × 30 × 20 cm), filled with water at a depth of 10 cm and at 22–25°C. After the last injection, the test was performed at intervals of 1, 3, and 8 days (NC, $n = 4$; MPTP $n = 4$). The swim time was scored as follows: 1 min of continual swimming, 3 points; mostly swimming with random floating, 2.5 points; floating time accounting for 50% of the total test time, 2 points; random swimming, 1.5 points; random swimming using hindlimbs and floating close to the periphery, 1 point.

2.3 | Traction behavior test

According to the method of Kuribara et al. (1977), a mouse was hung from the horizontal bar by the forelimbs; after confirming that the bar was well-grasped, the mouse was left hanging and observed by assessing the other limbs. Over a 10 s period, grasping the steel bar with both hindlimbs was recorded as 3 points, grasping with a single hindlimb was afforded 2 points, failure to grasp with both limbs but no falling was afforded 1 point, while falling was recorded as 0 points. The test was repeated three times at intervals of 1 min, and the average scores were considered. The test was performed at intervals of 1, 3, and 8 days after the last MPTP/vehicle injection (NC, $n = 4$; MPTP $n = 4$).

2.4 | Golgi staining and dendritic spine analysis

Briefly, brain slices were cut using a microtome to obtain 100-μm thick sections. The slides with tissue sections were immersed in distilled water twice for 3 min each. Then, 2 ml of solution-4, 2 ml of solution-5, and 6 ml of double-distilled water were mixed in a 12 ml staining jar. The tissue section slides were placed in this mixture, covered, and incubated for 10 min. After completion of the reaction, the sections were immersed in distilled water two times, for 4 min each. For gradient dehydration, the sections were placed in 50%, 70%, and 95% alcohol for 5 min, respectively. Subsequently, sections were dehydrated in anhydrous alcohol 3–4 times for 5 min each time. Golgi staining was performed following the Golgi-Cox OptimStain™ Kit instructions. The sections were then sealed with coverslips using an undiluted, highly concentrated neutral resin sealer, followed by evaporation and drying prior to microscopic examination. The results of Golgi staining of neurons were analyzed using ImageJ software (National Institutes of Health, Bethesda, MD).

For each mouse, one brain hemisphere was subjected to Golgi staining ($n = 8$; NC, $n = 4$; MPTP, $n = 4$). After the Golgi staining procedure, sections were observed under a 1000× objective (LEICA DM4 B) and analyzed using ImageJ (Witzig et al., 2020).

Next, 6–8 MSNs were selected for each hemisphere, and 3 dendrites were selected per MSN for accuracy. The following criteria were used to select MSNs randomly: well-impregnated neurons; clearly visible branches or other precipitates not obscured by other neurons; absence of visibly broken processes on the neuron; MSN visually resembled a typical medium spiny neuron (Clabough et al., 2021).

2.5 | IHC

Briefly, mouse hemispheres were fixed in 4% paraformaldehyde for more than one week after dehydration with 20% and 30% sucrose, respectively. Next, fixed specimens were cut into 25-μm thick brain slices using a microtome (LEICA CM 1950) and stained according to the following steps. (1) Brain tissue sections stored in paraformaldehyde at 4°C were removed and rinsed with 0.01 M phosphate-buffered saline (PBS) for 5 min (×3 times) to remove the OCT encapsulant. (2) For antigen repair, brain slices were placed in an antigen repair cassette containing citrate buffer (pH = 6.0), heated at 98°C for 15 min, and cooled to room temperature. (3) Next, the sections were rinsed with 0.01 M PBS for 5 min (× 3 times). (4) Subsequently, an appropriate amount of 0.3% Triton-X 100 solution was added dropwise for 20 min at room temperature to increase cell membrane permeability. (5) An endogenous peroxidase blocker (3% H_2O_2) was added dropwise and incubated for 15 min at room temperature to block endogenous peroxidase. (6) Sections were then rinsed with 0.01 M PBS for 5 min (× 3 times). (7) An appropriate amount of normal goat serum working solution was added dropwise, followed by incubation for 20 min at 37°C. (8) The serum was decanted. (9) Primary antibodies of rabbit origin (PSD-95 [1:100], Abmart, China; SNK [1:300], Abecpta, Suzhou, China; spar [1:300], Proteintech, Wuhan, China; TH1: 200, Abecpta, Suzhou, China) were prepared at indicated dilution ratios, and then added to brain slices (300 μl), followed by overnight incubation at 4°C. (10) The following day, the brain slices were removed from the refrigerator and rinsed for 5 min (×3 times) using 0.01 M PBS. (11) Next, the ready-to-use anti-rabbit secondary antibody (Albscience, Wuhan, China) was added dropwise, followed by incubation at room temperature for 1 h. (12) Sections were rinsed with 0.01 M PBS for 5 min (× 3 times). (13) Subsequently, DAB color development was performed. (14) For mounting, brain sections were transferred to gelatin-coated adhesive slides, and the number and groups were recorded using a pencil. (15) Slides were dehydrated using 50% alcohol (3 min), 75% alcohol (3 min), 85% alcohol (3 min), 95% alcohol (3 min), 95% alcohol (3 min), anhydrous ethanol (2 min), and anhydrous ethanol (2 min). (16) To achieve transparency, slides were treated with xylene (I) for 5 min and xylene (II) for 3 min. (17) Neutral gum was added dropwise to cover the prepared brain sections, followed by a coverslip to seal the section. (18) After air-drying in a ventilated place, prepared slides were observed under a light microscope and images were obtained, followed by statistical analysis.

We used the average optical density (AOD) to analyze IHC images. For each animal ($n = 8$; NC, $n = 4$; MPTP, $n = 4$), three sections were considered to calculate the AOD. The AOD was calculated after transforming the mean gray level using ImageJ. The calculation was performed as previously described (Peyvandi Karizbodagh et al., 2021).

2.6 | Statistical analysis

Data analyses were performed using GraphPad Prism, version 8 (GraphPad Software, Inc., La Jolla, CA, USA). Comparison between groups was performed using the *t*-test. Data are presented as mean ±standard error of the mean (SEM).

3 | Results

3.1 | MPTP caused DA cell loss in substantia nigra

Histological examinations were performed using IHC to assess DA cells in the substantia nigra of the middle brain, calculating the AOD of positive TH. The 8-day specimens were selected for analysis. Based on the AOD of positive TH (Figure 1a and b), the MPTP group presented smaller values

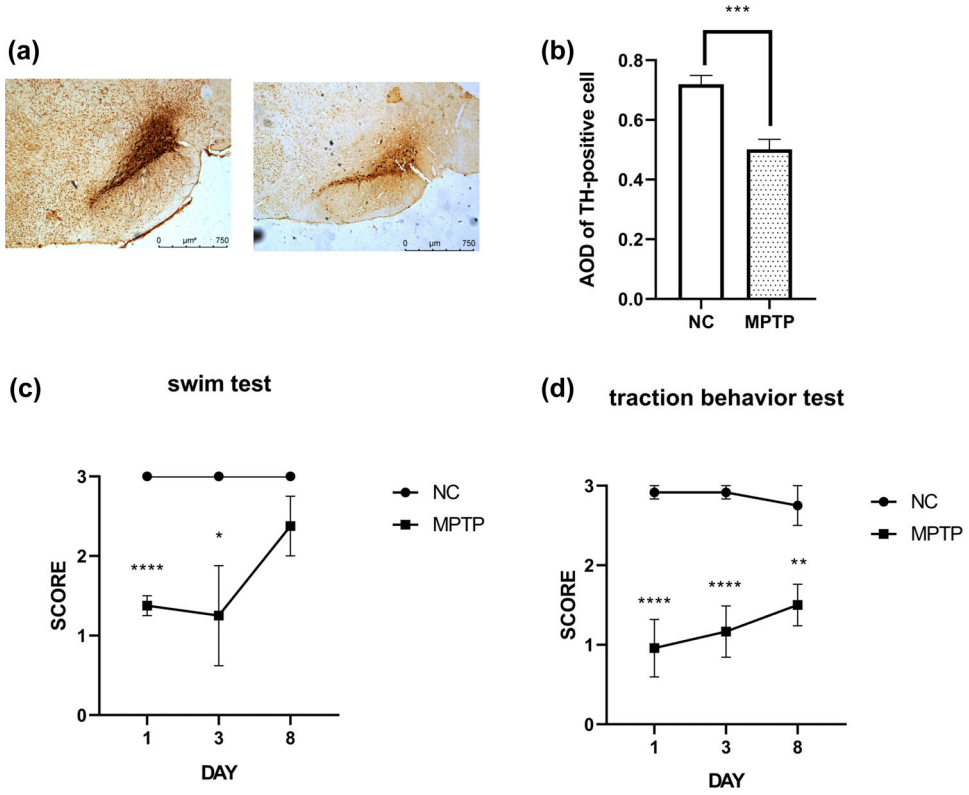

FIGURE 1 MPTP treatment triggers impairment of TH and motor function. (a) Substantia nigra was stained after 8 days to examine TH using IHC. (b) AOD of TH in substantia nigra. (c) Swim test score indicates that MPTP impacts motor function. (d) The traction behavior test score shows that MPTP affects motor function. Data are shown as the mean ± the standard error of mean $^{*}p < .0332$, $^{**}p < .0021$, $^{***}p < .0002$, $^{****}p < .0001$, $n = 4$ mice/group. AOD, average optical density; MPTP, 1-Methyl-4-phenyl-1,2,3,6-tetrahydropyridine; TH, tyrosine hydroxylase

than the NC group (NC: 0.7199 ± 0.02892 and MPTP: 0.5015 ± 0.03368 [$p = .0001$]). These findings indicated that MPTP could impair motor function and cause DA cell loss.

3.2 | MPTP treatment induced motor function impairment

Traction behavior and swim tests were performed to examine PD-related behaviors, and the experiments were recorded using a video camera. Volunteers randomly selected test videos for scoring. As shown in Figure 1c and d, MPTP treatment reduced scores in both experimental paradigms. Considering the 1-day group, MPTP significantly decreased behavioral scores in both test paradigms when compared with saline treatment in the NC group (traction behavior test, NC: 2.917 ± 0.08333 and MPTP: 0.9583 ± 0.3613 [$p = .000027$]; swim test, NC: 3±0 and MPTP: 1.375 ± 0.1250 [$p = .000013$]). In the 3-day test groups, test scores were found to be alleviated, but the MPTP group presented a lower score than the NC group (traction behavior test, NC: 2.917 ± 0.08333 and MPTP: 1.167 ± 0.3218 [$p = .000028$]; swim test, NC: 3 ± 0 and MPTP: 1.250 ± 0.6292 [$p = .031933$]). In the 8-day experiment, impaired traction behavior persisted (NC: 2.917 ± 0.08333 and MPTP: 1.5 ± 0.2611 [$p = .002239$]), whereas the swim test scores showed minimal differences between the two groups (NC: 3 ± 0 and MPTP: 2.375 ± 0.3750 [$p = .146630$]).

3.3 | MPTP treatment significantly reduced dendritic spine density in striatal MSNs

Golgi staining allows the examination of neurons and dendritic spines, facilitating the assessment of dendritic spine density. Considering the 8-day time point, Golgi staining revealed that the MPTP-treated group displayed reduced spine density in striatal neurons (Figure 2a and b) when compared with the NC mice (NC: 1.3±0.119/μm and MPTP: 0.7±0.678/μm [$p = .0095$]).

3.4 | MPTP reduced PSD-95 expression in striatal MSNs

PSD-95 is closely associated with synaptic framework. Accordingly, examining PSD-95 expression can help clarify synaptic remodeling in subcellular compartments. IHC can assess the PSD-95 expression semi-quantitatively. On examining 8-day specimens, the MPTP-treated group exhibited

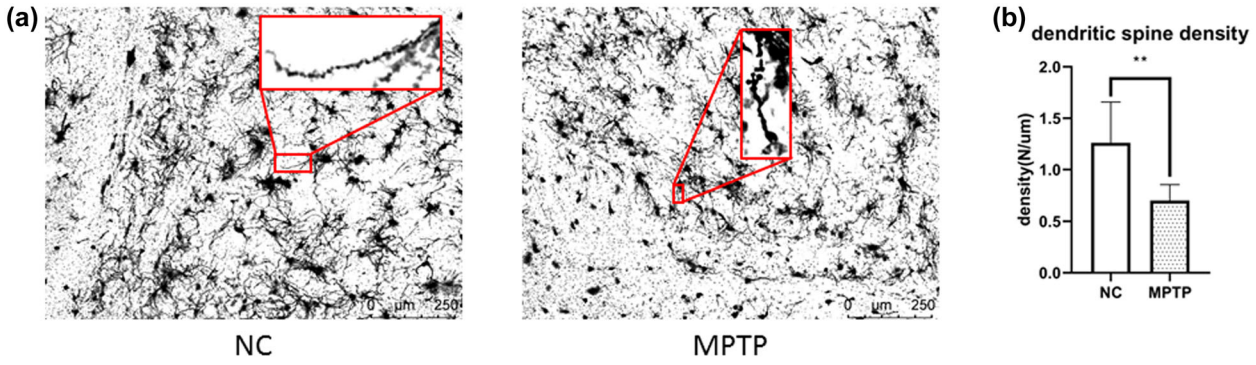

FIGURE 2 MSNs exhibit a decline in dendritic spine density. (a) Golgi staining shows that dendritic spine density is reduced in the 8-day experiment (b). ImageJ was used to analyze the dendritic spine density of MSNs. Data are shown as the mean ± the standard error of mean $^{**}p < .0021$, $n = 4$ mice/group. MSNs, medium spiny neurons

decreased PSD-95 expression in brain tissue sections when compared with the NC group (Figure 3a and b; NC: 0.44 ± 0.01691 and MPTP: 0.39 ± 0.01225 [$p = .0330$]).

3.5 | MPTP increased SNK expression in striatal MSNs

To examine SNK expression in vivo, fixed hemispheres were rapidly frozen and sectioned. Slices were stained to detect SNK using IHC, and the expression of SNK in striatal MSNs was analyzed using AOD values. On examining 8-day specimens, the MPTP-treated group showed increased SNK expression when compared with the NC group (Figure 3c and d; NC: 0.26 ± 0.001151 and MPTP: 0.35 ± 0.01786 [$p = .0016$]).

3.6 | MPTP decreased SPAR expression in striatal MSNs

As shown by IHC examination (Figure 3e and f), MPTP treatment markedly reduced SPAR expression in striatal MSNs, as determined by examining the 8-day specimens (NC: 0.21 ± 0.009120 and MPTP: 0.16 ± 0.003495 [$p = .0023$]).

4 | DISCUSSION

In the present study, we examined the underlying mechanism of MPTP-induced decline in dendritic spine density in striatal MSNs, as well as confirmed the potential role of the SNK-SPAR pathway in mediating this decline. In particular, we examined expression levels and morphology to elucidate the mechanism underlying the altered dendritic spine density. Following acute MPTP treatment, dendritic spine density of striatal MSNs was altered and found to decline, whereas expression of SNK was upregulated and SPAR expression was downregulated.

Accumulated data suggest that PD is a motor disorder associated with the loss of pigmented neurons of the substantia nigra and DA cell damage (Kumar, 2018). Herein, our results revealed DA cell loss in the substantia nigra of the middle brain and motor function impairment. Assessment of TH, a hallmark of DA cells, can indicate a significant decline in DA cells in the substantia nigra, as determined by IHC. Scores for both behavioral experiments were significantly decreased on examination 1 day after MPTP injection. Although the motor function recovered gradually, it remained lower than normal in the MPTP-treated group. Treatment with MPTP can stimulate a phenotype of PD, a DA-lesion model. Understanding the MPTP-induced damaging progress is critical to elucidate the progression of PD, primarily associated with reduced dendritic spine density. Establishing how MPTP treatment leads to decreased dendritic spines could help clarify the progression of PD and identify novel therapeutic targets.

Collectively, our findings correspond to previous studies and anticipation (Villalba et al., 2009; Weerasinghe-Mudiyanselage et al., 2021). The PD model has been successfully established in mice (Jenner, 2003); hence, determining the dendritic spine density of striatal MSNs and expression of SNK and SPAR can afford valuable insights.

Striatal MSNs relay multiple neurotransmitters between the cortex and substantia nigra, thereby impacting dendritic spine remodeling by depleting dopamine in the substantia nigra (Garcia et al., 2010; Neely et al., 2007). Administration of MPTP can damage DA cells in the substantia nigra and deplete dopamine input to the striatum (Dionisio et al., 2021). Herein, we postulated that MPTP reduced the dendritic spine density

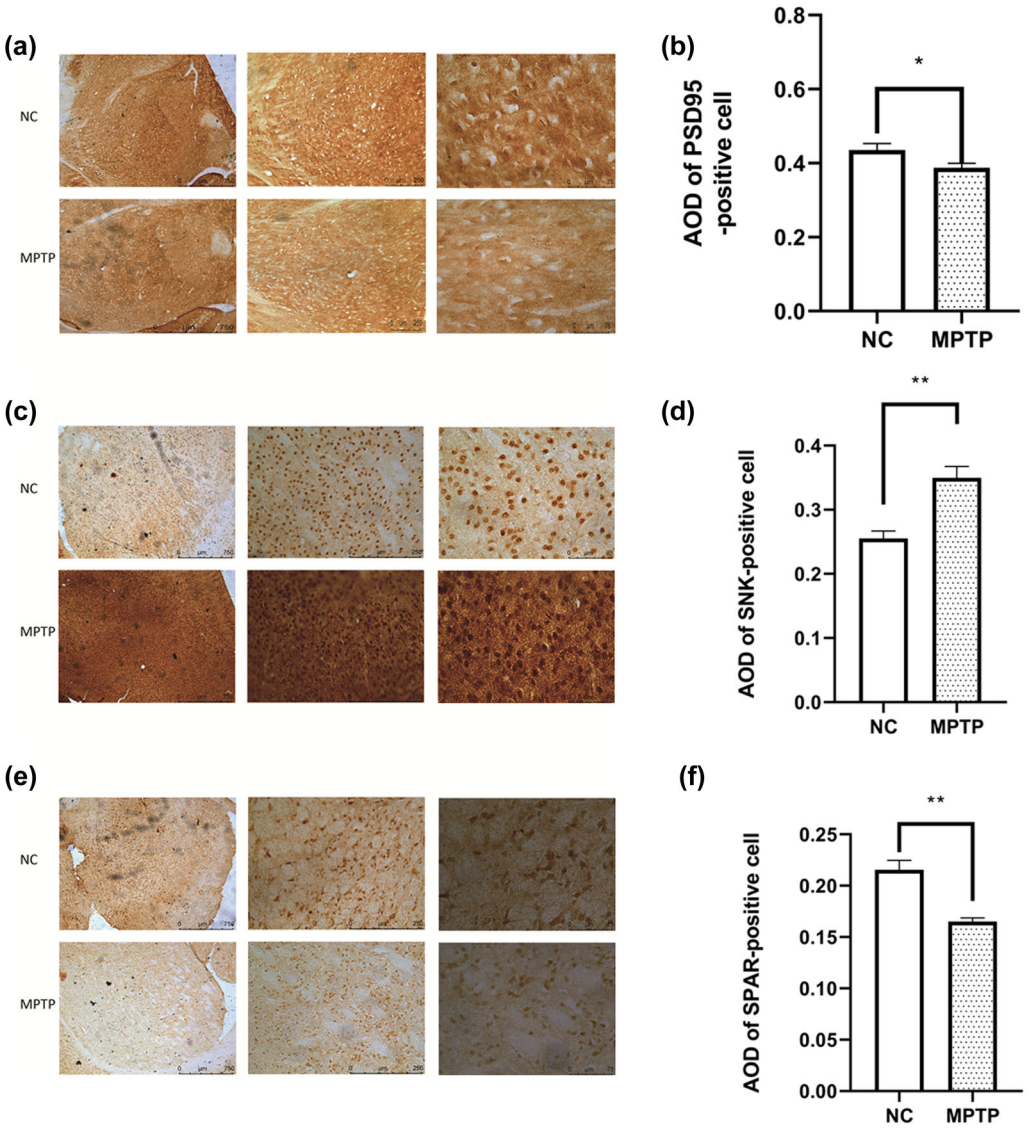

FIGURE 3 PSD-95 and SNK-SPAR were examined using IHC in 8-day experimental specimens. (a) MPTP treatment downregulated PSD-95 expression after 8 days. (b) AOD of PSD-95 positive cells, NC vs. MPTP. (c) MPTP treatment upregulated SNK expression after 8 days. (d) AOD of SNK-positive cells, NC vs. MPTP. (e) MPTP treatment downregulated SPAR expression after 8 days. (f) AOD of SNK-positive cells, NC vs. MPTP. Data are shown as the mean ± the standard error of mean $^{*}p < .0332$, $^{**}p < .0021$, $n = 4$ mice/group. AOD, average optical density; IHC, immunohistochemistry; MPTP, 1-Methyl-4-phenyl-1,2,3,6-tetrahydropyridine; SNK, serum-induced kinase; SPAR, and spine-associated Rap guanosine triphosphatase

of striatal MSNs. Compared with a previous study on long-time treatment (Witzig et al., 2020), the findings of the present study indicate that acute MPTP treatment could alter dendritic spine morphology in striatal MSNs. Furthermore, we found that the expression of PSD-95, a subcellular hallmark known to participate in synapse formation, was downregulated. Based on IHC results, the PSD-95 expression may indicate subcellular alterations in dendritic spines. To confirm the underlying mechanism, PSD-95-coupled molecules need to be examined. SNK and SPAR interact with moving PSD-95 to induce dendritic spine reduction (Pak & Sheng, 2003), facilitating the morphing of dendritic spines. Glutamate excitotoxicity was found to upregulate SNK expression and downregulate that of SPAR (Wu et al., 2007). Glutamate excitotoxicity can be attributed to glutamate overabundance following activation of the glutamate receptor. It can result in the death of overactivated neurons and the loss of dendritic spines (Fink & ScienceDirect, 2019). Herein, our findings were consistent with those previously reported: expression of the activated SNK-SPAR pathway was confirmed in vivo.

Moreover, the SNK-SPAR pathway may contribute to the loss of dendritic spines. The dendritic spine of striatal MSNs plays a crucial role in neurodegenerative diseases, especially its remodeling (Herms & Dorostkar, 2016). In Alzheimer's disease, activation of the SNK-SPAR pathway has been correlated with reduced dendritic spine density in the hippocampus (Gong et al., 2010). Based on our findings, we speculate that activating the SNK-SPAR pathway can reduce dendritic spines in the hippocampus (Chutabhakdikul & Surakul, 2013; Wu et al., 2007; Zhi et al., 2018) as well as

the striatum. This could clarify the MPTP-induced alterations in striatal dendritic density in MSNs. Notably, our findings demonstrate that the role of the SNK-SAPR pathway is not limited to the hippocampus and can be associated with PD.

The SNK reportedly participates in the phosphorylation of alpha-synuclein, a PD hallmark (Inglis et al., 2009). The SNK-SPAR pathway was found to be related to decreased dendritic spines. Further exploring the pathway may help to clarify the relationship between the reduced dendritic spines and PD progression. In addition to reducing dendritic spines density, this pathway may promote alpha-synuclein formation.

MPTP could depend on dopamine depletion to initiate the SNK-SPAR pathway of striatal MSNs. MSNs play a critical role in PD progression, and administration of MPTP can simulate PD-like symptoms accompanied by the loss of dendritic spines. Furthermore, activation of the SNK-SPAR pathway reduces dendritic spine density, and the SNK could also participate in alpha-synuclein formation. Accordingly, SNK could be critically associated with PD progression. Moreover, SNK might be associated with both pathways, that is, alpha-synuclein formation and reduction of dendritic spines. Future investigations should focus on expanding the role of the SNK-SPAR pathway in PD, as well as explore the relationship between the SNK-SPAR pathway and loss of dendritic spines and alpha-synuclein formation. Glutamate is a well-known neuro-excitotoxin, and glutamate can reportedly decrease dendritic spine density via SNK- SPAR pathway. We speculate that glutamate participates in activating the SNK-SPAR pathway. Future investigations need to explore molecules activated upstream of the SNK-SPAR pathway and determine the potential role of glutamate in this pathway.

5 | CONCLUSIONS

We successfully established a PD model in mice using MPTP in the present study and demonstrated motor function disorder and DA cell loss in histological specimens. Declining dendritic spine density of striatal MSNs was confirmed using Golgi staining, as determined by counting mature dendritic spines. IHC was used to examine the expression levels of SNK and SPAR. Our findings revealed that the SNK-SPAR pathway was activated in striatal MSNs. Accordingly, MPTP decreased the dendritic spine density of striatal MSNs, potentially by activating the SNK-SPAR pathway.

ACKNOWLEDGMENTS

This study was supported by Basic scientific research project of colleges and universities of Liaoning Provincial Department of Education (LJKZ1147).

DATA AVAILABILITY STATEMENT

This paper utilized original data not used in other publications. The data sets generated and/or analyzed in the present study are available from the corresponding author upon reasonable request.

CONFLICT OF INTEREST

The authors declare no conflicts of interest.

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How to cite this article: Su, Y., Jia, M., Yuan, S., Wang, C., Feng, J., & Zhang, Y. (2022). Acute MPTP treatment decreases dendritic spine density of striatal medium spiny neurons via SNK-SPAR pathway in C57BL/6 mice. *Synapse*, e22249. https://doi.org/10.1002/syn.22249