Right vagus nerve stimulation improves motor behavior by exerting neuroprotective effects in Parkinson's disease rats

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Background: Parkinson's disease (PD) is a common movement disorder disease. Left vagus nerve stimulation (LVNS) is a potential treatment option for PD. Compared with the left vagus nerve, the right vagus nerve is more closely connected with the midbrain dopaminergic neurons, which are the lesion locations of PD. However, whether right vagus nerve stimulation (RVNS) has a therapeutic effect on PD has not yet been studied. Therefore, in this study, we studied the therapeutic effect and underlying mechanism of RVNS using a PD rat model.

Methods: To establish the PD rat model, 8-week-old male Sprague-Dawley rats were intraperitoneally injected with rotenone for 21 days. The cuff electrodes were implanted into the right cervical vagal carotid sheaths of the rats. The right vagus nerve was continuously stimulated for 14 days using a radio stimulation system. Behavioral tests were performed before and after stimulation. Finally, tyrosine hydroxylase (TH), vesicular monoamine transporter 2 (VMAT2), and α-synuclein in the midbrain, including the substantia nigra (SN) and ventral tegmental area (VTA), were detected by immunofluorescence.

Results: A markedly lower distance traveled and rearing number was observed in the rotenone, rotenone + sham, and rotenone + RVNS groups compared to the vehicle group. After the stimulation days, the distance traveled and rearing number were both higher in the rotenone + RVNS group compared to the rotenone and rotenone + sham groups ($P<0.01$, $P<0.0001$). A remarkable increase in distance traveled and rearing number was observed in the rotenone + RVNS group after stimulation. TH expression in the vehicle group was significantly up-regulated than the other groups. RVNS markedly up-regulated TH expression level. A significantly higher expression of α-synuclein was observed in the rotenone, rotenone + sham, and rotenone + RVNS groups compared to the vehicle group. The expression of α-synuclein was lower in the rotenone + RVNS group compared to the rotenone and rotenone + sham groups. A markedly higher VMAT2 expression was observed in the vehicle group compared to other groups. RVNS significantly up-regulated VMAT2 expression.

Conclusions: The improved motor behavior and neuroprotective effects on the midbrain dopaminergic neurons in the PD rat model suggest that RVNS could be used as a potential treatment for PD.

Keywords: Parkinson's disease (PD); rat model; vagus nerve stimulation; right vagus nerve stimulation (RVNS)

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Introduction

Parkinson's disease (PD) is the second most common neurodegenerative disease after Alzheimer's disease and is also the most common movement disorder (1). The primary clinical symptoms of PD include bradykinesia, resting tremors, rigidity, and postural gait imbalance. The degeneration and loss of dopaminergic neurons in the midbrain and the aggregation of α-synuclein are the key factors contributing to the pathology of PD (2,3). Currently, multiple treatments for PD are available; however, no effective therapeutic strategies are presently available that could slow or prevent the progression of the disease (4,5). Therefore, additional research is necessary to develop new alternative treatment strategies for PD.

Recent studies have demonstrated an association between the vagus nerve and midbrain dopaminergic neurons. Research on the gut-brain neural circuits of rodents has revealed the existence of circuits between the vagus nerve and midbrain dopaminergic nuclei (6,7), validating studies that have shown PD to be gut-derived; i.e., the pathogenesis of PD is associated with the gastrointestinal nervous systems via vagus nerve propagation to the midbrain dopaminergic neurons (8-10). Previous studies have also shown that electrical stimulation of the vagus nerve affects the macromolecular structure and elemental composition in the midbrain dopaminergic regions of rats (11,12). Furthermore, vagus nerve stimulation can increase c-Fos expression in the dopaminergic neurons of the rat midbrain (13). A neuroimaging study conducted on the human brain showed that vagus nerve stimulation also activates the midbrain dopaminergic regions (14). These findings provide a research basis for the treatment of PD by vagus nerve stimulation.

Left vagus nerve stimulation (LVNS) has been approved by the Food and Drug Administration (FDA) for the treatment of epilepsy, depression, migraines, cluster headaches, pain, and obesity (15). Recently, LVNS has been explored for the treatment of PD. Farrand *et al.* first reported the therapeutic effects of LVNS in a PD rat model (16). Subsequently, various studies demonstrated that LVNS improves locomotion and exerts therapeutic effects on the midbrain dopaminergic neurons in PD rats (17-20). Clinical studies have also shown that non-invasive LVNS ameliorates the freezing of gait in PD patients (21-23).

Right vagus nerve stimulation (RVNS) has not been employed for the treatment of brain diseases due to its possible cardiac side effects (15). However, no studies have reported any incidences of cardiac side effects with RVNS. Han *et al.* used optogenetics to activate the ganglion neurons of the vagus nerve, and their results revealed that the right vagus, not the left, induced dopamine release in the midbrain (6). Another study compared the effects of RVNS with LVNS on the midbrain dopaminergic system, and the results suggested that RNVS preferentially activated the midbrain dopaminergic system (13). These studies suggest that the right vagus nerve is more closely connected with the midbrain dopaminergic neurons than the left vagus nerve. In addition, the pathogenesis of PD is associated with the gastrointestinal nervous systems via vagus nerve propagation to the midbrain dopaminergic neurons. Hence, RVNS could be involved with the pathogenesis of PD, which might become a potential treatment option for PD. However, no studies have shown that RVNS has a therapeutic effect on PD, and therefore, there is a pressing need to investigate the therapeutic effect of RVNS on PD.

In this study, we performed RVNS on a PD rat model using a radio stimulation system. The therapeutic and neuroprotective effects of RVNS on the PD rat model were evaluated by analyzing the motor behavior changes and immunofluorescence assay. We present the following article in accordance with the ARRIVE reporting checklist (available at https://atm.amegroups.com/article/view/10.21037/atm-22-5366/rc).

Highlight box

Key findings

- Therapeutic effect of RVNS on PD rats.

What is known and what is new?

- VNS especially RVNS activates dopaminergic neurons in the rat midbrain.
- RVNS improves motor behavior of PD rats by exerting protective effect on midbrain dopaminergic neurons.

What is the implication, and what should change now?

- RVNS could be explored as a potential treatment for PD. Further studies are needed to explore the additional underlying mechanisms of RVNS in the treatment of PD.

Methods

Animals

Eight-week-old (280–300 g) adult male Sprague-Dawley rats were used in this study. The rats were randomly divided

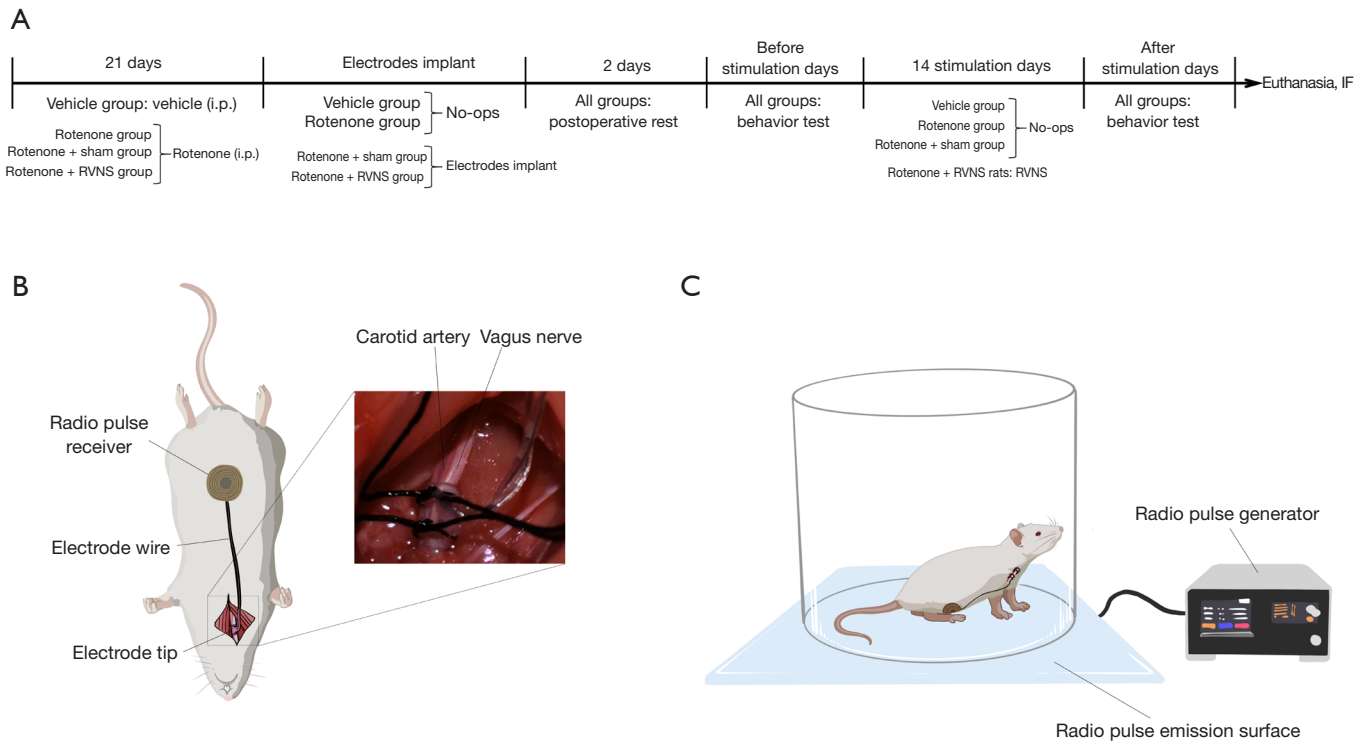

Figure 1 Experimental design. (A) Rotenone was injected intraperitoneally for 21 days to establish the PD rat model. The cuff electrodes were then implanted in the rotenone + sham and rotenone + RVNS groups. Behavioral tests were performed on all of the rats after 2 days of postoperative rest. RVNS was performed for 14 days on rats in the rotenone + RVNS group. Following stimulation, all of the rats underwent behavioral tests. Finally, the rats were euthanized to perform immunofluorescence. (B) The cuff electrode consisted of a radio pulse receiver, electrode wire, and electrode tip. The radio pulse receiver was implanted subcutaneously in the abdomens of the rats. The electrode wire was threaded through the subcutaneous tunnel to the neck, and the cuff electrode tip was wrapped around the right vagus carotid sheath. (C) The radio stimulation system consisted of a radio pulse generator and a radio pulse emission surface. RVNS, right vagus nerve stimulation; IF, immunofluorescent; PD, Parkinson's disease.

into four groups (with six rats in each group) based on the type of treatment, such as rotenone use, the implantation of electrodes, or RVNS: vehicle (n=6), rotenone (n=6), rotenone + sham (n=6), and rotenone + RVNS (n=6). All of the rats were purchased and raised at the Animal Center of Ningxia Medical University, and were housed in a specific pathogen-free environment in a 12 h/12 h light/dark cycle at 23–25 ℃ constant room temperature and maintained in groups (three rats per cage) with access food and water ad libitum. Animal experiments procedures were approved by the Animal Research Ethics Committee of Ningxia Medical University (No. 2022-G196), in compliance with Animal Research Ethics Committee of Ningxia Medical University guidelines for the care and use of animals. A protocol was prepared before the study without registration.

Establishment of the PD rat model

Rotenone (99.65% purity; MCE, China) was used to establish the PD rat model. Rotenone powder was dissolved in dimethyl sulfoxide (DMSO; MCE, China) and diluted with normal saline to obtain the target solution of 1.5 mg/mL rotenone and 1% DMSO. Rotenone (1.5 mg/kg/day) was intraperitoneally administered to the rats for 21 days (*Figure 1A*) (24). Rats in the vehicle group were intraperitoneally injected with a 1.0 mL/kg/day vehicle (1% DMSO and 99% saline) (*Figure 1A*).

Implantation of electrodes

The cuff electrode consisted of a radio pulse receiver,

electrode wire, and electrode tip (*Figure 1B*). The rats were anesthetized by isoflurane (3–5% induction, then 2–3% maintenance) inhalation and placed in the supine position on a constant temperature table to maintain their body temperature. An incision was then made on the right side of the rat's neck. The radio pulse receiver was implanted subcutaneously in the abdomen, and the electrode tip emerged from the neck through the chest subcutaneously (*Figure 1B*). The cervical muscles were bluntly separated, and the vagal carotid sheath was between the sternocleidomastoid and sternohyoid muscles. The cuff electrode was then wrapped around the vagal carotid sheath (*Figure 1B*), and the incision was sutured. The electrodes were implanted into rats from the rotenone + sham and rotenone + RVNS groups (*Figure 1A*).

RVNS

A radio stimulation device (Kedou Brain Computer Technology Co., LTD., China) was used to perform RVNS. The device consisted of a radio pulse generator and a radio pulse emission surface (*Figure 1C*). RVNS was performed upon completion of the first behavioral tests (*Figure 1A*). The conventional vagus nerve stimulation parameters were used for stimulation, i.e., a pulse frequency of 30 Hz, a current intensity of 1.0 mA, a pulse width of 500 microseconds, and stimulation for 30 seconds at 5 minutes intervals (19). The rotenone + RVNS group received stimulation for 14 days (*Figure 1A*). The electrodes were also implanted into rats from the rotenone + sham group but no stimulation was performed (*Figure 1A*).

Open field test

In our study, the open field test was used to measure the motor behavior of rats before and after the RVNS stimulation days (*Figure 1A*). The rats were acclimatized to the test environment 1 hour before conducting the test and then placed in the center of the open field box with the back of the rat facing the investigator and allowed to explore freely for 5 minutes. The experiments were then conducted, and the rats were maintained in quiet and low-light conditions. A video tracking system was used to record the exploratory behavior of the rats, and Smart3.0 software (Panlab, Spain) was used to record and analyze the data. The entire open field box was cleaned with 75% ethanol and dried before testing the next rat. The statistical parameters analyzed were the distance traveled by the rat and the rearing number.

Immunofluorescent staining and image analysis

After the last open field test, all of the rats were euthanized with isoflurane and transcardially perfused with 4% paraformaldehyde dissolved in phosphate buffer. The rat brains were quickly removed and placed in perfusate overnight. The next day, the brains were transferred to a 30% sucrose solution for cryopreservation. 10-μm thick coronal sections of the frozen brain were made at the midbrain site comprising the substantia nigra (SN) and ventral tegmental area (VTA). Immunofluorescence was performed using antibodies mouse tyrosine hydroxylase (TH, 1:1,000, AB150659, Abcam, UK), rabbit α-synuclein (1:500, GB11404, Servicebio, China), and rabbit vesicular monoamine transporter 2 (VMAT2, 1:1,000, AB70808, Abcam, UK). The sections were blocked in 10% normal donkey serum for 1 hour and then incubated with primary antibodies for TH, α-synuclein, and VMAT2 overnight. Thereafter, the sections were incubated for 1 hour with secondary antibodies (cyanine 5-conjugated goat anti-mouse 1:200, GB27301, Servicebio, China; fluorescein isothiocyanate-conjugated goat anti-rabbit, 1:500, GB22303, Servicebio, China). Images were subsequently captured using a laser scanning confocal microscope (Leica DM6, Wetzlar, Germany).

The mean gray value of the images was measured to quantify the expression levels of TH, α-synuclein, and VMAT2 in the midbrain dopaminergic neurons using ImageJ software (NIH, USA). The range of dopaminergic neurons labeled by TH in the SN and VTA was used to draw the region of interest manually by raters blinded to the study, who also measured the mean gray value (13).

Statistical analysis

Statistical analyses were performed using analysis of variance (ANOVA) by GraphPad Prism 8.0.2 (GraphPad Software, La Jolla, CA, USA). All data were represented as the mean ± standard error of the mean (SEM). $P<0.05$ was considered statistically significant (**$P<0.01$, ***$P<0.001$, ****$P<0.0001$, ns: no statistical significance).

Results

Effects of RVNS on motor behavior

All of the rats were subjected to open field tests before and

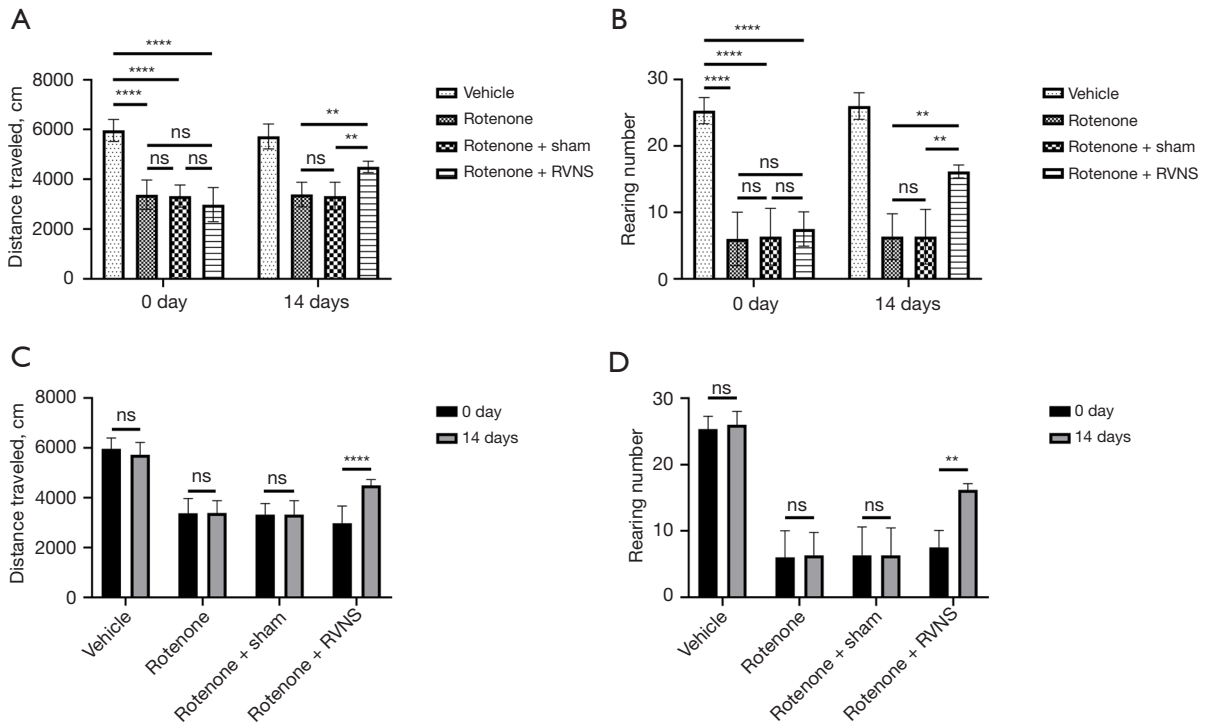

Figure 2 RVNS improves motor behavior in PD rats. (A) The distance traveled by the rats before and after the stimulation days. (B) The rearing number before and after the stimulation days. (C) Comparison of the distance traveled by the rats before and after the stimulation days in each group. (D) Comparison of the rearing number before and after the stimulation days in each group. One-way and two-way ANOVA were applied for statistical analysis. **P<0.01; ****P<0.0001. ns, no statistical significance; sham, sham surgery; RVNS, right vagus nerve stimulation; PD, Parkinson's disease; ANOVA, analysis of variance.

after the stimulation days, and the distance traveled and rearing number were used to evaluate their motor behavior.

A markedly lower distance traveled and rearing number was observed in the rotenone, rotenone + sham, and rotenone + RVNS groups compared to the vehicle group (*Figure 2A,2B*). Before the stimulation days, no significant difference in motor behavior was observed between the rotenone, rotenone + sham, and rotenone + RVNS groups (*Figure 2A,2B*). After the stimulation days, the distance traveled and rearing number were both higher in the rotenone + RVNS group compared to the rotenone and rotenone + sham groups (**P<0.01, ****P<0.0001; *Figure 2A,2B*). Even after the stimulation days, no notable difference in motor behavior was observed between the rotenone and rotenone + sham groups (*Figure 2A,2B*).

A remarkable increase in distance traveled and rearing number was observed in the rotenone + RVNS group after stimulation (*Figure 2C,2D*). However, the difference in motor behavior in the vehicle, rotenone, and rotenone + sham groups before and after the stimulation days was not statistically significant (*Figure 2C,2D*).

Effects of RVNS on TH and α-synuclein expression

Immunofluorescence was performed to study the effect of RVNS on TH and α-synuclein expression in the SN and VTA of the midbrain (*Figure 3A,3B*).

A significantly lower TH expression was observed in the rotenone, rotenone + sham, and rotenone + RVNS groups compared to the vehicle group (*Figure 3C*). No notable difference in TH expression was observed between the rotenone and rotenone + sham groups (*Figure 3C*). Also, a markedly higher TH expression was observed in the rotenone + RVNS group compared to the rotenone and rotenone + sham groups (*Figure 3C*).

Moreover, a significantly higher expression of α-synuclein was observed in the rotenone, rotenone + sham, and rotenone + RVNS groups compared to the vehicle group (*Figure 3D*). The difference in α-synuclein expression between the rotenone and rotenone + sham groups

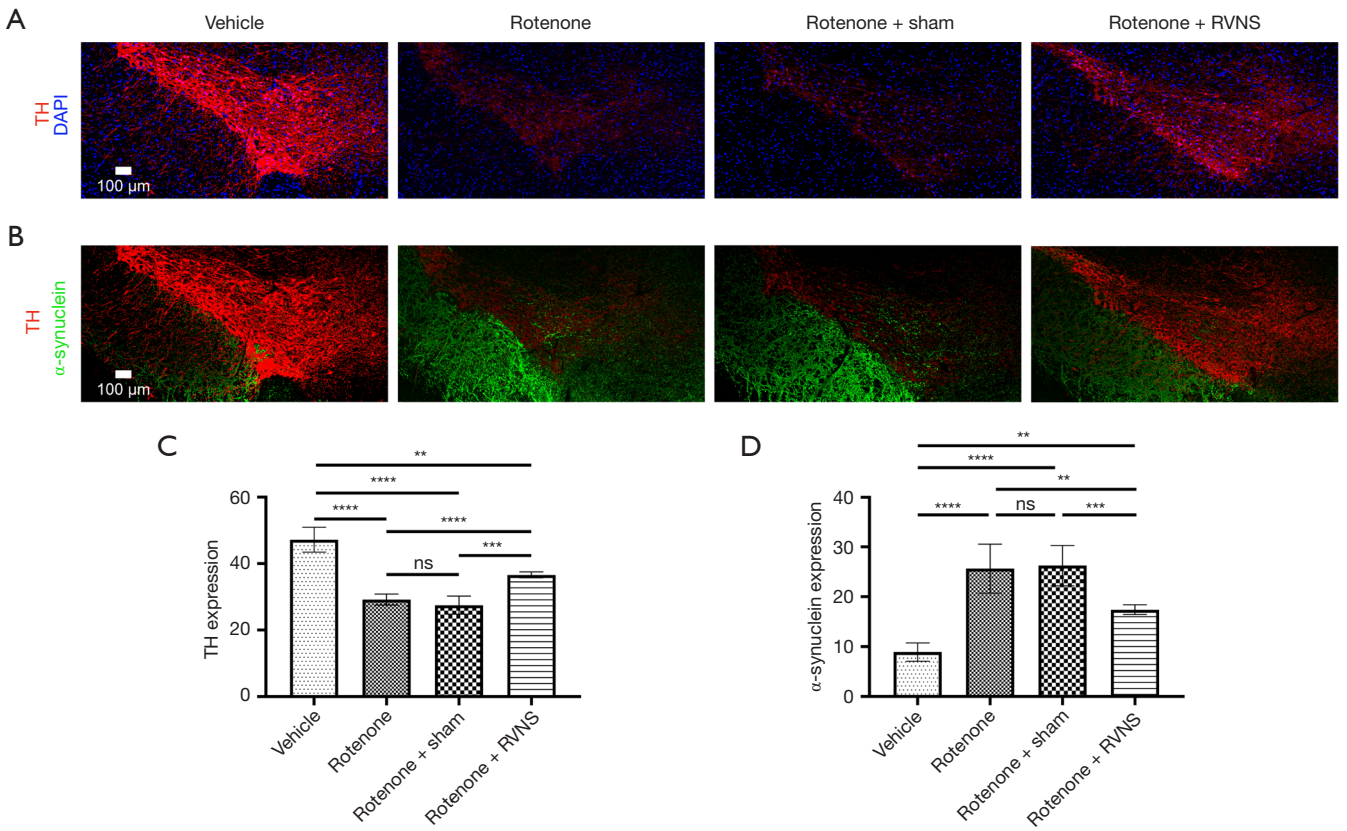

Figure 3 RVNS increases TH expression and decreases α-synuclein expression in PD rats. (A) Red TH and blue DAPI immunofluorescence images in the SN and VTA. (B) Immunofluorescence images of the red TH and green α-synuclein in the SN and VTA. (C,D) The image analysis results of TH (C) and α-synuclein (D) immunofluorescence. One-way ANOVA was used for statistical analysis. **P<0.01; ***P<0.001; ****P<0.0001. TH, tyrosine hydroxylase; DAPI, 4',6-diamidino-2-phenylindole; sham, sham surgery; RVNS, right vagus nerve stimulation; ns, no statistical significance; PD, Parkinson's disease; SN, substantia nigra; VTA, ventral tegmental area; ANOVA, analysis of variance.

(*Figure 3D*) was not statistically notable. The expression of α-synuclein was lower in the rotenone + RVNS group compared to the rotenone and rotenone + sham groups (*Figure 3D*).

Effect of RVNS on VMAT2 expression

TH was used to label the SNs and VTAs of the rats (*Figure 4A*,*4B*). Immunofluorescence was performed to assay the expression of VMAT2 in the SN and VTA of the rat midbrains (*Figure 4C*).

A markedly higher VMAT2 expression was observed in the vehicle group compared to the rotenone, rotenone + sham, and rotenone + RVNS groups (*Figure 4D*). No significant difference in VMAT2 expression was observed between the rotenone and rotenone + sham groups (*Figure 4D*). Also, the VMAT2 expression was considerably higher in the rotenone + RVNS group compared to the rotenone and rotenone + sham groups (*Figure 4D*).

Discussion

In this study, we investigated the therapeutic effects and mechanism of RVNS in a PD rat model. The improvement in motor behavior of the PD rat models after RVNS could be attributed to the neuroprotective effect of RVNS.

The clinical diagnosis of PD is based on three core motor symptoms; i.e., the requisite bradykinesia coupled with the presence of at least one of the following two symptoms: resting tremor or rigidity (25). Hence, the performance of the PD rat model was evaluated based on the reduction in activity, slow movement, tremor, and postural gait

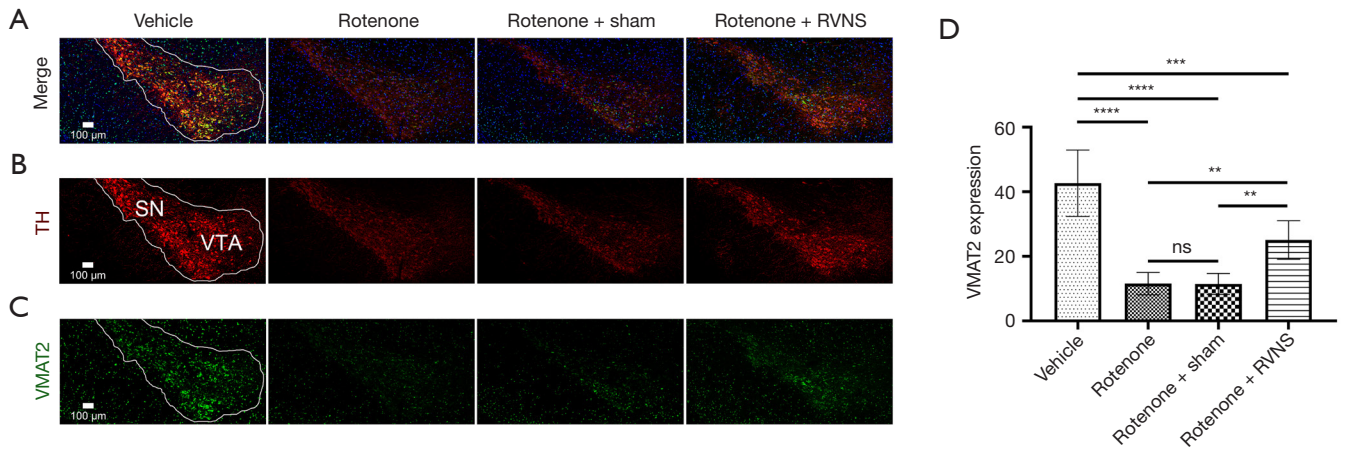

Figure 4 RVNS increases VMAT2 expression in PD rats. (A,B) Red TH-labeled dopaminergic neurons in the SN and VTA. (C) Immunofluorescence images of green VMAT2 in the SN and VTA. (D) The image analysis results of VMAT2 immunofluorescence. One-way ANOVA was used for statistical analysis. **P<0.01; ***P<0.001; ****P<0.0001. sham, sham surgery; RVNS, right vagus nerve stimulation; TH, tyrosine hydroxylase; SN, substantia nigra; VTA, ventral tegmental area; VMAT2, vesicular monoamine transporter 2; ns, no statistical significance; PD, Parkinson's disease; ANOVA, analysis of variance.

imbalance (24). The main feature of the PD rat model was reduced activity (26). In our study, the locomotion of PD rats was measured by evaluating the distance traveled by the rats and the rearing number in the 5-minute open field test. Our results showed an increase in the distance traveled and the rearing number in PD rats following RVNS treatment. This represents an improvement in motor ability, highlighting the therapeutic effects of RVNS in PD rats.

TH is a rate-limiting enzyme for dopamine synthesis, decreased TH expression in surviving dopaminergic neurons is observed in PD (27,28). A previous study showed that an increase in TH levels in surviving dopaminergic neurons alleviates the motor symptoms of PD (29). The accumulation of α-synuclein is a hallmark feature of PD. In PD, the degeneration and loss of dopaminergic neurons in the brain are closely related to the abnormal accumulation of α-synuclein. The increased expression of α-synuclein in the midbrain dopaminergic neurons is positively correlated with the severity of movement disorders in PD (30). The translocation of dopamine to synaptic vesicles by VMAT2 prevents the formation of neurotoxic derivatives of dopamine in the cytosol (31-33). Low VMAT2 expression degenerates the dopaminergic neurons, highlighting the neuroprotective role of VMAT2 (34). The TH, α-synuclein, and VMAT2 expression levels reflect the cytoactivity of dopaminergic neurons. In our study, the alleviation of motor dysfunction was observed in the RVNS-treated PD rats. Furthermore, the immunofluorescence results revealed TH and VMAT2 expression was higher and α-synuclein expression was lower in the midbrain dopaminergic neurons of the PD rats treated with RVNS compared to those that did not receive treatment. This suggests that RVNS exerts a neuroprotective effect on PD, which is possibly the underlying therapeutic mechanism of RVNS.

In this study, the vagus nerve was not separated from the carotid artery to avoid an ischemic vagal paralysis associated with this procedure. Instead, cuff electrodes were wrapped around the vagus carotid sheath, as described previously (18). Rotenone, a mitochondrial complex I inhibitor, was used to establish the PD rat model. The PD rat model, established using rotenone, displayed more typical pathological changes and symptoms of PD compared with other neurotoxin PD rat models (35). In our study, the motor behavior results and TH, α-synuclein, and VMAT2 expression patterns indicated the successful establishment of the PD rat model using rotenone. However, establishing the PD rat model using rotenone was labor-intensive and time-consuming. Also, the rats were susceptible to death if the rotenone suspension was inadequately mixed before injecting the rats. Furthermore, the rotenone + sham group was used in our study to demonstrate that the results were not influenced by the surgery or electrode implantation.

The vagus nerve is the largest, longest, and most special cranial nerve, with a wide range of distribution. The vagus nerve has anatomical asymmetry and central vagal circuits asymmetry (6,36). RVNS and LVNS exert different effects

on the brain (13). Various studies have focused on the effects of LVNS on brain diseases; however, not much attention has been paid to the use of RVNS. Recently RVNS has been used to treat heart failure but has not yet been applied for the treatment of brain diseases. Its possible cardiac side effects have likely discouraged the use of RVNS for the treatment of brain disease. However, the adverse effects of RVNS on heart conditions, such as bradycardia, are based on the fact that, anatomically, the right vagus nerve innervates the sinus node, which has no experimental basis. Hence, there is no direct evidence that RVNS can cause cardiac accidents (13,37).

Several limitations have to be acknowledged in this study. First, current studies (13,30) have shown the association between vagus nerve and the midbrain dopaminergic neurons. Nevertheless, the correlation between vagus nerve and neuron network in other locations has not been extensively studied and validated. Consequently, more relevant investigations remain to be carried out to evaluate the role of RVNS in affecting the dopaminergic neurons in alternative locations. Second, these preliminary findings obtained in the present study should be further tested in other PD animal models, such as MPTP-induced PD model. More in-depth studies and clinical investigations are needed to test and verify our findings.

Conclusions

In conclusion, we investigated the therapeutic effects of RVNS on PD rats and the underlying mechanisms associated with its therapeutic effects. Our results demonstrated that RVNS improved the motor behavior of PD rats. The underlying mechanism associated with the therapeutic effect of RVNS on PD rats could be attributed to the neuroprotective effect of RVNS on dopaminergic neurons in the midbrain. Further studies are needed to explore the additional underlying mechanisms of RVNS in the treatment of PD and compare the efficacy of RVNS and LVNS.

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Footnote

Reporting Checklist: The authors have completed the ARRIVE reporting checklist. Available at https://atm.amegroups.com/article/view/10.21037/atm-22-5366/rc

Data Sharing Statement: Available at https://atm.amegroups.com/article/view/10.21037/atm-22-5366/dss

Conflicts of Interest: All authors have completed the ICMJE uniform disclosure form (available at https://atm.amegroups.com/article/view/10.21037/atm-22-5366/coif). TS reports funding support from the National Natural Science Foundation of China (No. 82260282). The other authors have no conflicts of interest to declare.

Ethical Statement: The authors are accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved. Animal experiments were performed under a project license (No. 2022-G196) granted by Animal Research Ethics Committee of Ningxia Medical University, in compliance with Animal Research Ethics Committee of Ningxia Medical University guidelines for the care and use of animals.

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