Based on variability parameters analysis of autonomic function in rats with Parkinson's disease induced by rotenone

Yi Yu
School of Medical Engineering, Xinxiang Medical University
Xudong Gao
School of Medical Engineering, Xinxiang Medical University
Mengke Wang
School of Medical Engineering, Xinxiang Medical University
Miaoqing Lei
School of Medical Engineering, Xinxiang Medical University
Yilin Liu
School of Medical Engineering, Xinxiang Medical University
Peiqi Chen
School of Medical Engineering, Xinxiang Medical University
Xuewei Zhao
School of Medical Engineering, Xinxiang Medical University
Yifan Feng
School of Medical Engineering, Xinxiang Medical University
Heshun Hu
School of Medical Engineering, Xinxiang Medical University
Yanxiang Niu
School of Medical Engineering, Xinxiang Medical University
Chong Wang
Zhenpeng Shao
School of Medical Engineering, Xinxiang Medical University
Yang Wu
Yu Zhu
Junlin Mu
The Second Affiliated Hospital of Xinxiang Medical University
Zongya Zhao (zhaozongya_paper@126.com)
School of Medical Engineering, Xinxiang Medical University

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Abstract

Background: Parkinson's disease is a common neurodegenerative disease, and while early detection of cardiovascular symptoms may aid in early diagnosis, there are no definitive markers to assess cardiovascular autonomic damage in Parkinson's disease.

Methods: Twenty Sprague Dawley rats were randomly assigned to the experimental (n=12) and control (n=8) groups, and a model of Parkinson's disease was created in the experimental group by stereotaxic injection of rotenone into the substantia nigra compacta and ventral tegmental area. Telemetry implantation was carried out after successful modeling. Equal dosages of saline were injected into control animals at the same places. In both groups of rats, ECG, blood pressure, core temperature, and activity were recorded using a Data Science International implantable physiological signal telemetry device. The time domain, frequency domain, and non-linear analysis were used to examine the blood pressure and heart rate variability of the two groups of rats.

Results: The experimental group had higher Detrended Fluctuation Analysis (DFA) of blood pressure signals (systolic, diastolic, and mean arterial pressure), mean arterial pressure, normalized high frequency power (nHF) than the control rats ($p<0.05$). The experimental group had lower sample entropy (SampEn) of blood pressure signals (diastolic and mean arterial pressure), root mean squared successive differences (RMSSD), normalized lower frequency power (nLF) and total power than the control group ($p<0.05$). However, the standard deviation, coefficient of variation, and continuous variation in the linear analysis of continuous blood pressure signal between the two groups were not statistically significant ($p>0.05$).

Conclusion: The rats in the rotenone model had significant autonomic dysfunction, and non-linear analysis approaches such as detrended fluctuation analysis and sample entropy were able to discern the diseased condition of the rats more sensitively while processing the continuous blood pressure data.

1. Introduction

Parkinson's disease (PD) is a slowly progressive neurodegenerative disease that has a significant impact on the health of middle-aged and elderly people[1,2]. It is characterized by the progressive loss of dopaminergic neurons in the substantia nigra and the production of Lewy bodies in residual cells[3,4]. Motor symptoms such as rigidity, myotonia and static tremor, as well as non-motor symptoms associated with autonomic impairment, such as cardiovascular diseases, upright hypotension, sleep disorders, and constipation, are the most common clinical manifestations of Parkinson's disease[5–7]. Autonomic dysfunction affects 80 percent of Parkinson's disease patients, resulting in a variety of symptoms[8,9]. The quality of life suffers, and in severe cases, sudden death may occur [10–12]. Non-motor symptoms of Parkinson's disease have been found to occur up to ten years earlier than motor symptoms, with cardiovascular autonomic function changes being the most common non-motor symptom, and the high prevalence of cardiovascular disease observed in patients with Parkinson's disease proving a correlation

between the two, and early detection of cardiovascular autonomic disorders plays a role in the early diagnosis of PD [11,13]. Understanding the changes in cardiovascular autonomic function in Parkinson's disease would thus aid clinical diagnosis and early intervention in the disease.

Cardiovascular health is usually demonstrated by a normal heart rate and blood pressure, but recording of animal's cardiovascular data is often disturbed by other factors such as anesthesia and activity, and telemetry is able to record the relevant physiological signals in awake rats, thus minimizing the influence of other factors[14]. The cardiovascular autonomic nerves mainly consist of sympathetic and vagal nerves, which are antagonistic to each other and interact with each other[15]. When the cardiovascular autonomic nerve is damaged, it can cause cardiovascular dysfunction and induce cardiovascular diseases, especially arrhythmias, and the analysis of variability parameters can achieve sensitive capture of abnormalities in cardiovascular autonomic nerve function[16,17]. Blood pressure variability (BPV) and heart rate variability (HRV), which is a measure for assessing the degree of cardiovascular variability by extracting beat-by-beat blood pressure or per beat Heartbeat time series, are two of the variability parameters[18–21]. The autonomic nervous system and stress reflexes can be evaluated by converting recorded blood pressure and ECG data into HRV and BPV. The use of variable parameters allows for a quantitative assessment of the cardiovascular autonomic nervous system's functioning[22].

However, investigations on cardiovascular autonomic abnormalities in PD rats are less common in animal models than studies on motor symptoms, and it is not clear how the cardiovascular autonomic changes and continuous blood pressure fluctuations in the stereotaxic injection model of rotenone rats. As a result, we used variability parameters to assess the impairment of cardiovascular autonomic function in Parkinson's rats induced by stereotaxic rotenone injection, with the goal of identifying effective indicators to distinguish the status of cardiovascular autonomic in rats and providing a series of tools to aid in the clinical diagnosis and evaluation of Parkinson's disease.

2. Results

2.1 Modeling

Twelve rats were molded using a two-point injection of rotenone, with one dying during surgery, one dying from postoperative infection, and seven successfully molded. The unsuccessful rats were sacrificed under the influence of an overdose of anesthesia. In the control group, no one died. After successful modeling, the rats demonstrated irritability, hair erection, gait stiffness, and rotation in place during feeding, as well as stable rotation as shown in Fig. 1 induced by apomorphine (1 ml/kg) and greater than 7 revolutions per minute.

2.2 Behavioral experiments

The rat rotating bar experiment assessed the locomotor ability and coordination of rats, and the results are shown in Fig. 2. The results showed that when compared to the control group (n = 7; 162.03 ± 2.71s),

the experimental group (n = 6; 57.14 ± 33.40s; t = 6.819; p < 0.001***) had a significantly shorter locomotor time, indicating that PD rats had significant motor impairment.

2.3 Morphological examination

Morphological analysis primarily demonstrates the immunofluorescence of TH-positive neurons in the substantia nigra compacta; TH is a dopaminergic neuron specific staining parameter; the greater the degree of TH positivity, the greater the number of dopaminergic neurons present. Figure 3 depicts the immunofluorescence results. The number of TH-positive neurons in the substantia nigra of rats induced by rotenone was significantly reduced when compared to the contralateral brain area, indicating that the modeling was successful.

2.4 Rat physiological status comparison

Table 1 summarizes the physiological status of normal and rotenone-induced PD rats. The experimental group's mean arterial pressure (n = 6; 100.8 ± 59.11 mmHg) was higher than the control group's (n = 7; 77.76 ± 5.93 mmHg; t=-2.493; p = 0.030*), and the difference was statistically significant (p < 0.05), while the differences in the remaining indexes were not statistically significant (p > 0.05).

Table 1
physiological status of normal and rotenone-induced PD rats.

	n	HR/bpm	SBP/mmHg	DBP/ mmHg	MAP/mmHg	Temperature /°C	Activity
Control Group	7	331.24 ± 32.77	103.26 ± 12.83	77.76 ± 5.93	90.03 ± 6.50	38.02 ± 0.44	0.05 ± 0.05
Experimental Group	6	341.75 ± 78.71	115.48 ± 10.09	88.55 ± 11.35	100.85 ± 9.11*	37.64 ± 0.45	0.07 ± 0.07

Note: Compared with the control group *p < 0.05 ,**p < 0.01,***p < 0.001. HR: Heart rate SBP: Systolic Blood Pressure DBP: Diastolic Blood Pressure MAP: Mean Arterial Pressure.

2.5 HRV time-frequency analysis for the two groups of rats

Table 2 shows the results of HRV time-frequency analysis for the two groups of rats. The RMSSD (n = 6; 2.15 ± 0.55ms; t = 2.384; p = 0.036*), nLF (n = 6; 0.17 ± 0.09; t = 7.285; p = 0.048*), and all power (n = 6; 2.15 ± 1.05 ms^2; t = 2.828; p = 0.016*) were lower than those of the control rats (n = 7; 4.20 ± 2.05 ms) (n = 7; 0.43 ± 0.28). The nHF (n = 6; 0.83 ± 0.09; t = 7.285; p = 0.048*) of rats in the experimental group was higher than that of the control group (n = 7; 0.57 ± 0.28) and the difference was statistically significant (p < 0.05), but the differences in the remaining indexes were not (p > 0.05).

Table 2
HRV time-frequency analysis

	n	SDNN/ms	RMSSD/ms	nLF/nu.	nHF/nu.	LF/HF	All power/ms^2
Control Group	7	5.70 ± 2.10	4.20 ± 2.05	0.43 ± 0.28	0.57 ± 0.28	1.25 ± 1.25	11.33 ± 7.84
Experimental Group	6	4.00 ± 1.38	2.15 ± 0.55*	0.17 ± 0.09*	0.83 ± 0.09*	0.21 ± 0.14	2.15 ± 1.05*

Note: Compared with the control group *p < 0.05. SDNN: Standard Deviation of Normal to Normal; RMSSD: root mean square of successive differences; nLF: Normalized low frequency; nHF: Normalized High frequency; LF/HF: low frequency/ High frequency.

2.6 Linear analysis of BPV in two groups of rats

The results of BPV linear analysis are shown in Table 3. the standard deviation, coefficient of variation, and continuous variation in the linear analysis of continuous blood pressure signal between the experimental group and the control group were not statistically significant (p > 0.05).

Table 3
Linear analysis of BPV

		SD/mmHg			CV/mmHg			SV /mmHg		
Group	n	SBP	DBP	MAP	SBP	DBP	MAP	SBP	DBP	MAP
Control Group	7	6.1 ± 1.04	5.52 ± 0.88	5.81 ± 0.91	0.06 ± 0.01	0.07 ± 0.01	0.07 ± 0.01	3.32 ± 0.93	3.14 ± 0.88	3.16 ± 0.85
Experimental Group	6	5.54 ± 1.05	5.94 ± 1.8	5.95 ± 1.74	0.05 ± 0.01	0.07 ± 0.03	0.06 ± 0.02	3.26 ± 0.94	3.04 ± 0.78	3.09 ± 0.86

Note: Compared with the control group *p < 0.05. SD: standard deviation; CV: Coefficient of variation; SV: successive variation; SBP: Systolic Blood Pressure; DBP: Diastolic Blood Pressure; MAP: Mean Arterial Pressure.

2.7 BPV nonlinear analysis

Figure 4 summarizes the parameters of nonlinear analysis of BPV in two groups of rats. Figure 4A shows that the sample entropy values of diastolic blood pressure (n = 6; 2.28 ± 0.16; t = 4.247; p = 0.001***) and mean arterial pressure (n = 6; 2.27 ± 0.16; t = 2.384; p = 0.036*) in the continuous blood pressure signal of rats in the experimental group were lower than those of rats in the control group (n = 7; 2.78 ± 0.24) (n = 7; 2.7 ± 0.45) and was statistically significant (p < 0.05), however, the sample entropy characteristic of systolic blood pressure in the experimental group (2.3 ± 0.16) was lower than that of systolic blood pressure sample entropy in the experimental rats (n = 6; 2.45 ± 0.48; t = 0.791; p = 0.453), which was not statistically significant (p > 0.05). As shown in Fig. 4B, the continuous blood pressure signals in the

experimental group of rats for systolic (n = 6; 0.95 ± 0.05; t=-3.468; p = 0.005**), diastolic (n = 6; 0.95 ± 0.05; t=-2.576; p = 0.032*), and mean arterial pressure (n = 6; 2.27 ± 0.16; t = 2.359; p = 0.047*) were higher than those of control rats (n = 7; 0.80 ± 0.10) (n = 7; 0.82 ± 0.12) (n = 7; 0.81 ± 0.11) in the detrended fluctuation analysis values, and all were statistically different ($p < 0.05$).

3. Discussion

In this study, we calculated the variability parameters of a rotenone-induced Parkinson's disease model to assess the impairment of cardiovascular autonomic function in the model of PD. Parkinson's disease is a complex condition that affects multiple organs throughout the body, with cardiovascular symptoms standing out among the non-motor symptoms[26,27]. Given the severity of cardiovascular-related symptoms, we used long-term ECG with Continuous blood pressure as a monitoring method, and then calculated variability parameters (blood pressure variability, heart rate variability) to determine the health status of the PD rat model. We found no significant changes in physiological status in the rotenone PD model, including no changes in heart rate, temperature, or mobility, indicating that unilateral stereotactic modeling of rotenone has a minor effect on physical function and does not cause serious harm to rats. Furthermore, central dopamine loss is similar to the pathophysiological relationship of Parkinson's disease in humans, and the effect of substantia nigra and striatal dopaminergic neuron loss on cardiovascular injury can be better explored by central rotenone injection.

HRV is used to assess the balance of sympathetic and parasympathetic nerves in cardiac autonomic nerves and to quantify cardiovascular autonomic function by calculating subtle changes in sinus rhythm per beat interval[28,29]. The severe imbalance in autonomic function reflected by the PD model's HRV is reflected by a decrease in total power of HRV, a decrease in RMSSD in the time domain, a decrease in the low-frequency component, and an increase in the high-frequency component in the frequency-domain analysis. RMSSD is the root mean square of the interpolated RR interval in time-domain analysis and is the primary indicator of heart parasympathetic innervation[30–32]. According to Table 2, the RMSSD of the experimental rats was lower than that of of the control rats, implying that the parasympathetic innervation of the heart in PD rats was affected compared to normal rats, with less volatility in the RR interval variation and more regular heartbeat rhythms, and this regularity represents to some extent the heart's reduced regulation. The signal was Fourier transformed in the frequency domain into several specific frequency bands, with the high frequency component (HF) reflecting parasympathetic activity and the low frequency component (LF) containing both sympathetic and parasympathetic effects. The normalization of the spectral components was represented on the autonomic nervous system balance in cardiac innervation. The decrease in nLF and increase in nHF in the experimental group reflected changes in sympathetic and parasympathetic innervation in the rotenone model rats, with parasympathetic innervation becoming more dominant and sympathetic innervation decreasing. The findings are consistent with the pathological changes in the autonomic nervous system caused by Parkinson's disease discovered by Ariza et al[33] in a 6-OHDA animal model and by Alonso et al [13] in a clinical study, namely that PD causes significant time and frequency changes in HRV, a decrease in total power

reflecting overall autonomic indicators, and a decrease in RMSSD reflecting parasympathetic innervation. The possible mechanism is an imbalance of cardiac sympathetic and parasympathetic innervation caused by the central nerve's influence on the vagus nerve dorsal nucleus, which is reached via vagal intestinal diffusion by the Lewy bodies[34–37]. Furthermore, the rats in the experimental group had low activity and dirty hair, confirming the autonomic innervation imbalance. In this study, telemetry was used to record long-duration ECG signals, which reduced interference in special states such as anesthesia and improved data accuracy when compared to other animal tests in which 5-min ECG signals were collected under anesthesia.

A combination of neural and humoral factors influence changes in arterial blood pressure, which can be easily measured and quantified. Blood pressure signals contain a wealth of physiological and pathological data. Traditional animal blood pressure measurements can only obtain systolic and diastolic blood pressure data at a specific point in time, but in this experiment, a pressure catheter was inserted into a blood vessel and continuous blood pressure was obtained using telemetry technology, allowing for long-term blood pressure monitoring. Circadian rhythm disturbances, upright hypotension, supine hypertension, and other symptoms are all symptoms of Parkinson's disease[38,39]. The model group had a statistically significant increase in mean arterial pressure, but not in systolic or diastolic blood pressure, which we conjecture is due to blood pressure fluctuations caused by changes in circadian rhythm in rats due to Parkinson's disease. Further research will focus on the analysis of circadian rhythm changes in the rotenone model rats and will extend the time of day when signals are collected. Table 3 was primarily used to examine the variability of the continuous blood pressure signal. It has been demonstrated that two people with similar blood pressure can have different blood pressure volatility, and that continuously monitoring blood pressure to obtain blood pressure variability sequences can be used as a hidden indicator independent of other cardiovascular risk factors[22,40]. The standard deviation, coefficient of variation, and continuous variation of continuous blood pressure in experimental and control rats did not show significant differences in this study, but non-linear algorithms like sample entropy and detrended fluctuation analysis were used to sensitively identify changes in blood pressure fluctuations in the two groups of rats. This is in line with the findings of Fares et al[41], who found that nonlinear analysis methods can more acutely capture indicators of subtle changes in intravascular pressure than traditional blood pressure variability analysis of standard deviation and coefficient of variation. In general, both sample entropy and detrended fluctuation analysis revealed changes in the blood pressure signal's nonlinear parameters, i.e., subtle changes in vascular pressure in PD rats were monitored in the pathological state, quantifying the biological system's instability in the pathological state[42]. The entropy value measures the irregularity of biological signals; the lower the entropy value, the more regular the signal, and the higher the entropy value, the more disordered the signal[43,44]. The relationship between reduced complexity and increased time series in biological systems is linked to the diseased state, with healthy organisms having higher complexity, that is, higher entropy values. This was corroborated in our investigation, where the sample entropy of diastolic and mean arterial pressure was significantly lower in the experimental group of rats than in the control group, while the sample entropy of systolic pressure showed no statistical differences. We hypothesized that Parkinson's disease mainly

affects the volatility of diastolic blood pressure, i.e. the probability of generating new information in the diastolic blood pressure time series of PD rats is lower than that of normal controls, based on the clinical association of Parkinson's disease fatigue symptoms with lower levels of diastolic blood pressure[45]. The DFA values of systolic, diastolic, and mean arterial pressure in the continuous blood pressure signal of PD rats increased, showing that the continuous blood pressure signal's long time series correlation and predictability improved. Blood flow fluctuations in PD rats tended to be regular and predictable, reflecting the rotenone rat model's minor changes in hemodynamics. To summarize, we used a nonlinear approach to determine the variability of blood pressure fluctuations in PD rats versus normal rats, and future research will focus on changes in dynamic blood pressure in the early and late stages of dopaminergic neuronal degeneration, allowing us to investigate trends in blood pressure changes over time.

The current study confirmed that the rotenone stereotaxic model group experienced significant autonomic impairment. This was primarily seen as a reduction in total power in heart rate variability and an increase in high frequency components in normalized spectral analysis. In the absence of significant differences in arterial blood pressure and linear parameters, this study evaluated the variability of continuous blood pressure signals using nonlinear parameters such as sample entropy and detrended fluctuation analysis, perceptibly differentiating the changes in blood flow status in Parkinson's disease rats. This study used telemetry to record the cardiac and blood pressure signals of rats while they were awake, and it analyzed the functional changes of the cardiovascular and autonomic nervous systems in the Parkinson's disease state more precisely. Further research should concentrate on changes in blood pressure signals as the disease progresses, changes in nonlinear dynamics of blood pressure signals, and changes in cardiac autonomic function in the early stages of the disease. Simultaneously, in collaboration with clinical practice, we will design prospective studies to provide more reliable theoretical support for the study of cardiovascular autonomic function impairment in Parkinson's disease.

4. Materials And Methods

4.1 Experimental animals

20 Specific Pathogen Free (SPF) grade Sprague Dawley rats, weighing 280–310 g, were purchased from Henan Skibes Biotechnology Co. The rats were placed in a constant temperature and ventilation grade animal room at 26℃ for one week for acclimatization, given standard feed and drinking water for feeding, and fed freely.

4.2 Materials and Instruments

Rotenone and apomorphine were purchased from Sigma Corporation (St. Louis, Missouri, USA). The implantable physiological signal telemetry system, bioadhesive, and fiber membrane were purchased from Data Sciences International Corporation(St. Paul, Minnesota, USA) and the implant sub-model was HD-S11-F0. Isoflurane, small animal stereotaxic instrument, and animal anesthesia machine were purchased from RWD (Life Science Co., Shenzhen, China).

4.3 Model preparation

A rat model of Parkinson's disease was created by randomly dividing rats into experimental (n = 12) and control (n = 8) groups, and a two-point stereotaxic injection method was used. The rat head was prepared and secured to the stereotaxic apparatus before a 1-cm opening was cut into the scalp with a surgical blade to remove the connective tissue between the skin and the skull and locate the fontanelle. The stereotaxic atlas[23] was used to locate the ventral tegmental area (AP + 4.9 mm, ML + 1.0 mm, DV-8.5 mm) of the substantia nigra compacta (AP + 5.0 mm, ML + 2.0 mm, DV-8.2 mm), and holes were drilled at the above two points without penetrating the meninges using a cranial drill. After slowly lowering the microinjection needle into the target brain area at a speed of 1 mm/min, 2 ul rotenone was injected into each spot, and the needle was left for 10 minutes before being slowly withdrawn. At each point, 2ul of saline was injected into the control rats. Following surgery, the skin was sutured after the wound was cleaned. For six days, the amount of 0.5 ml of penicillin were administered daily to prevent infection. After 4 weeks of recovery, the rats were injected intraperitoneally with 1mg/kg apomorphine, and it was observed whether the rats were connected head to tail and rotated in place, among other things. The number of rotations in 30 minutes was recorded, and continuous rotations faster than 7r/min were considered successful modeling[24,25].

4.4 Telemetry implantation

Following successful modeling and control rats, telemetry implantation was performed in 13 rats. Isoflurane was inhaled to anesthetize, and the abdominal skin was prepared and placed on a heating pad covered with sterile sheets for the procedure. For the placement of the implanted substrate, a 2–3 cm incision was made along 1 cm below the xiphoid, and two 0.5 cm incisions were made below the left abdomen and above the right thorax for the placement of the electrocardiographic leads. After exposing the abdominal cavity, the abdominal aorta and vena cava were separated, the blood pressure catheter was inserted 1.5 cm into the abdominal aorta, and the vascular incision was connected using 3M bioadhesive and fibrous membrane. Finally, the main body of the implant and the cardiac electrode were sutured to the muscle tissue, and the abdominal cavity was closed. For six days, the amount of 0.5ml of penicillin were administered daily to prevent infection. The rats' condition was monitored on a daily basis following the operation.

4.5 Signal acquisition and extraction

The signals were gathered 7 days following the implantation operation in an electromagnetically shielded environment. The rats were placed on the signal receiving board, the telemetry sub-switch was turned on with a magnet, and the ECG, blood pressure, core temperature, and activity of the rats in the awake state in the experimental and control groups were recorded for 30 minutes after the rodents regained calmness. The raw blood pressure and ECG signals were preprocessed using MATLAB software, which included removing the interfering signals of the industrial frequency, band-pass filtering to eliminate the interference of the EMG signals, and removing the linear trend, among other things. After preprocessing, the R peaks of the ECG signals were detected using the time domain difference approach to obtain heart

rate variability sequences, and the blood pressure signals were acquired using the forward difference method to obtain continuous blood pressure sequences. Finally, the three spline interpolation approach was used to remove the trend. The signal was evaluated in the time domain, frequency domain, and nonlinearity after preprocessing. Standard deviation of RR intervals (SDNN) and root mean squared successive differences (RMSSD) of HRV; standard deviation(SD), coefficient of variation (CV), and continuous variation (SV) of continuous blood pressure were calculated in the time domain. Frequency domain analysis was performed using the welth method to calculate the spectral components of heart rate variability and the area under each frequency band: vLF (0-0.16 Hz), LF (0.16–0.6 Hz), and HF (0.6-3 Hz). The nonlinear parameters of the blood pressure signal were calculated using sample entropy and detrended fluctuation analysis.

4.6 Behavioral experiments

Using a rotating bar fatigue apparatus to evaluate the motor function of rats. The rat is placed on a roller and starts to move in the opposite direction as the roller rotates. The rat becomes less coordinated and falls in a short period of time when its motor function is impaired. Each channel has an infrared monitoring device that is triggered when the rat falls, allowing the time and distance of movement to be recorded. The rat moved for 3 minutes at a speed of 15 revolutions per minute. When the rat fell or stopped moving, the experiment was over. Each group was repeated three times with a 15-minute interval between them, and the movement data was averaged over the three times.

4.7 Morphology experiments

Following successful signal recording, two rats were chosen at random for morphological analysis. The brain was perfusion for extraction, then soaked in paraformaldehyde, dehydrated in sucrose, paraffin embedded, and tissue sections were made. Finally, the dense brain area of the substantia nigra was stained for tyrosine hydroxylase, and morphological changes were observed under fluorescence microscopy.

4.8 Statistical

For statistical analysis, SPSS 22.0 software was used, and the results were expressed as mean standard deviation ($\bar{x}\pm s$). A t-test for independent samples was used to compare groups, and a P value of less than or equal to 0.05 was considered statistically significant.

Declarations

Ethical Approval and Consent to participate

The experiment was approved by the Ethics Committee of Xinxiang Medical College.

Availability of data and materials

The datasets used and analyzed in the current study are available from the corresponding author upon reasonable request.

Conflicts of interest

The authors declare that they have no competing interests.

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Authors' contributions

YY and XG designed and performed the study. MW,ML,YL,PC helped the rat's surgeries and signal recordings. XZ,YF,SH contributed to the data analysis . YN,CW,ZS,YW contributed by compiling the chart.ZZ,JM contributed by correcting the article.XG contributed to the major writing of manuscript. All authors reviewed the manuscript.

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Figures

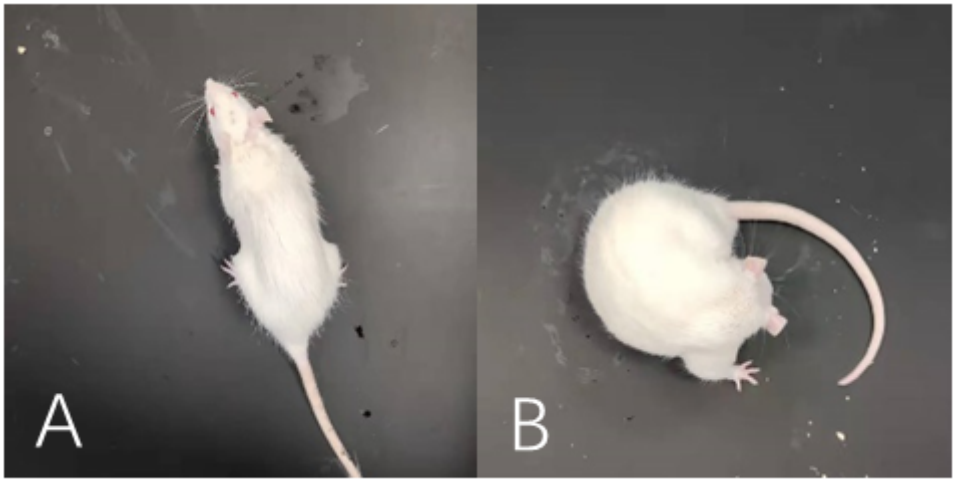

Figure 1

Parkinson's disease rats model establishment. (A) A unsuccessful PD rat model. (B) A successful PD rat model that depicts contralateral rotation (left).

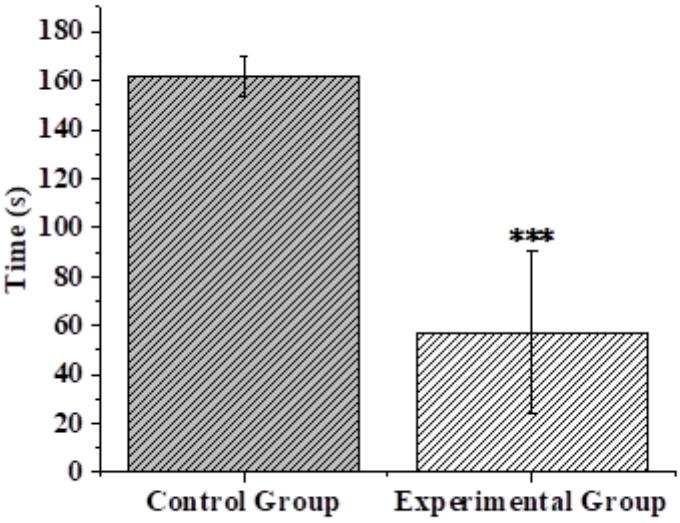

Figure 2

Exercise time of rat rotating bar experiment.

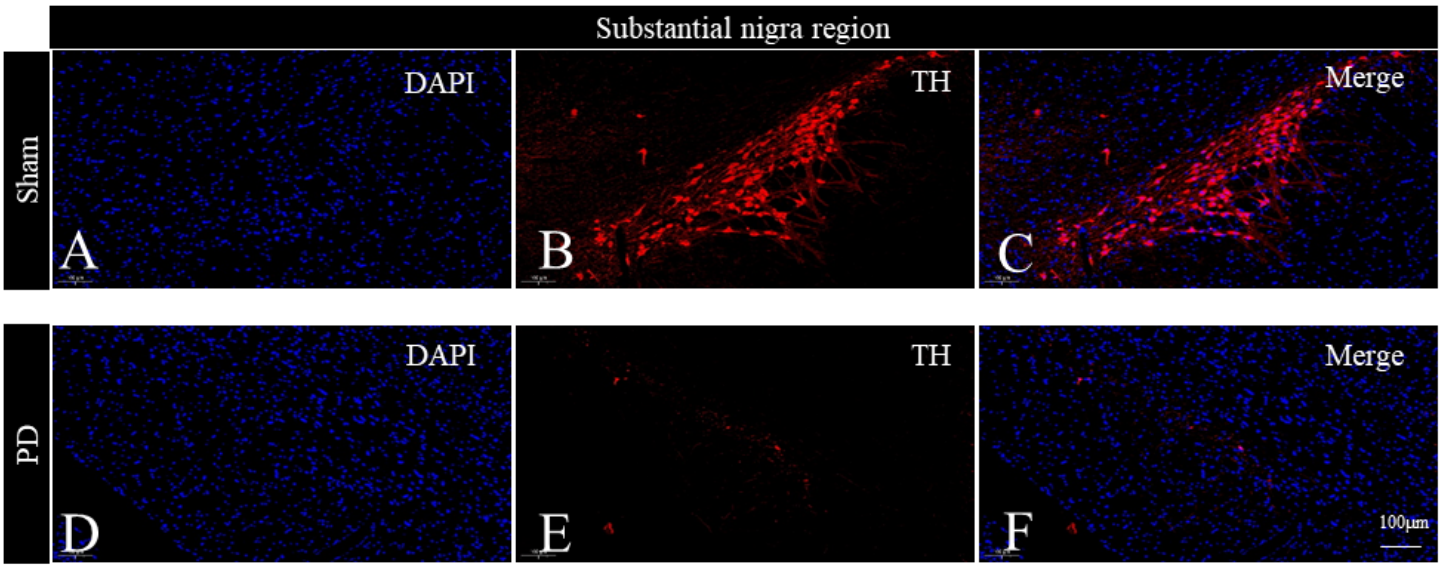

Figure 3

Immunofluorescence of the substantia nigra. (A-C) Immunofluorescence of the substantia nigra in the left cerebral hemisphere. (D-F) Immunofluorescence of the substantia nigra in the right cerebral hemisphere (Damaged side).

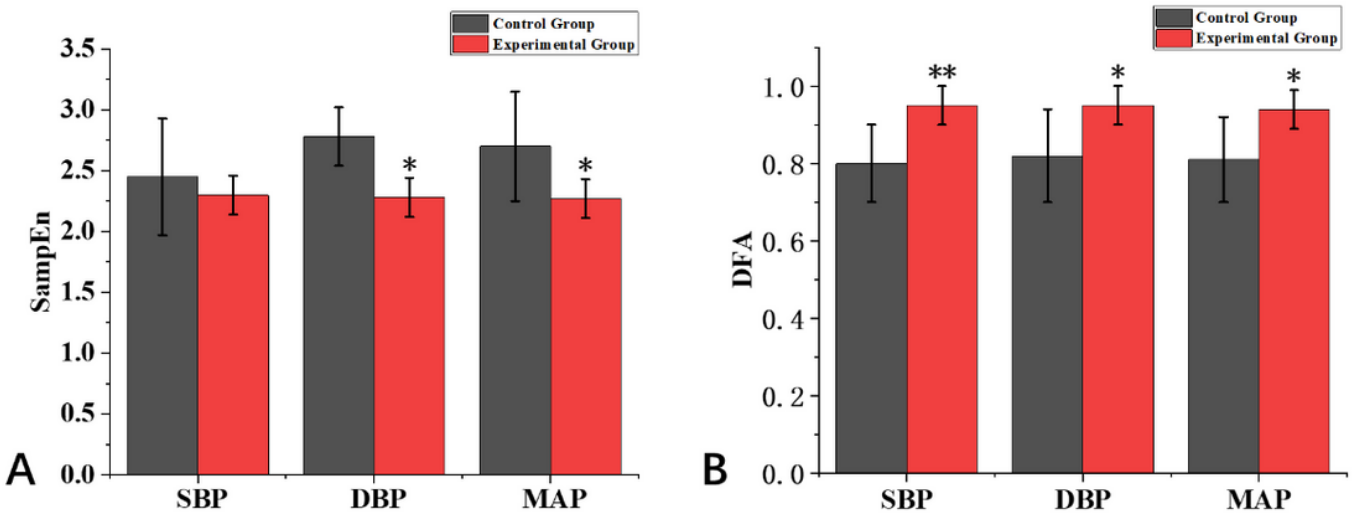

Figure 4

BPV nonlinear analysis: Characteristic values of continuous blood pressure signals in normal rats and PD rats. (A) Sample entropy (B) detrended fluctuation analysis. SBP: Systolic Blood Pressure DBP: Diastolic Blood Pressure MAP: Mean Arterial Pressure.