

# Evaluation of the early response and mechanism of treatment of Parkinson's disease with L-dopa using $^{18}\text{F}$ -fallypride micro-positron emission tomography scanning

YI-HUA FA<sup>1</sup>, JIAN-QIANG NI<sup>2</sup>, XIAO-JIN WU<sup>3</sup>, JIA-QING TAN<sup>4</sup> and YI-WEI WU<sup>1,4</sup>

<sup>1</sup>School of Radiation Medicine and Protection, Medical College of Soochow University, Suzhou, Jiangsu 215000; Departments of <sup>2</sup>Neurology, <sup>3</sup>Hematology and <sup>4</sup>Nuclear Medicine, The First Affiliated Hospital of Soochow University, Suzhou, Jiangsu 215006, P.R. China

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**Abstract.** The aim of the present study was to investigate the use of  $^{18}\text{F}$ -fallypride micro-positron emission tomography (micro-PET) imaging in the evaluation of the early therapeutic efficacy of L-dopa in the treatment of Parkinson's disease (PD) and the underlying mechanism.  $^{18}\text{F}$ -fallypride was synthesized and its specific binding with dopamine (DA) receptors in normal mouse brain was studied. Following the establishment of a mouse model of PD, the animals were divided into normal control, PD model and L-dopa treatment groups. General behavior, swimming test, locomotor activity counts, transmission electron microscopy, immunohistochemical analysis, high performance liquid chromatography-electrochemical detection and  $^{18}\text{F}$ -fallypride micro-PET imaging were used to study intergroup differences and the correlation between the changes of striatal uptake of  $^{18}\text{F}$ -fallypride and the therapeutic efficacy. The general behavioral features of PD model mice were similar to the clinical symptoms of PD patients and were alleviated after treatment. The swimming time, locomotor activity and frequency of standing posture of PD model mice were lower than those of the control mice, but had no difference from those of the control mice after L-dopa treatment. The number of tyrosine hydroxylase-positive neurons and the striatal contents of glutathione peroxidase, superoxide dismutase, DA and its metabolites 3,5-dihydroxyphenylacetic acid and homovanillic acid in the PD group were lower than those in the control group, but were significantly improved following the treatment; the significant reduction in DOPAC/DA and HVA/DA ratios post treatment suggested that the rate of DA metabolism decreased significantly. The striatal

malondialdehyde content in the PD group increased compared with that in the control group, but was reduced after L-dopa treatment. Micro-PET imaging indicated that the uptake of  $^{18}\text{F}$ -fallypride in the mouse striatum of the PD group was lower than that of the control group and was significantly increased after the treatment. The mechanism of treatment of PD with L-dopa in mice may involve increasing the number of TH-positive cells and DA receptor levels, as well as reducing the rate of DA metabolism; such changes can be noninvasively observed *in vitro* by  $^{18}\text{F}$ -fallypride imaging.

## Introduction

Parkinson's disease (PD) is the most common movement disorder, which occurs worldwide and increases in incidence with age. In China, the prevalence and incidence of PD are 2 per 100,000 population and 797 per 100,000 person-years (1). The clinical symptoms of PD mainly manifest as extrapyramidal movement disorders, such as tremor, rigidity, bradykinesia and a mask-like, expressionless face, which seriously affect the patients' quality of life (2). The main pathological characteristics of PD are the progressive degeneration of dopaminergic neurons in the substantia nigra pars compacta (SNpc) and the appearance of eosinophilic inclusions (Lewy bodies), which leads to a reduction of dopamine (DA) levels and extrapyramidal movement disorder; the underlying mechanism may be associated with, for example, oxidative stress, mitochondrial dysfunction or protein misfolding (3,4). When the symptoms begin, 50-80% of dopaminergic neurons have already been destroyed (5). A worldwide problem is that good drugs and effective methods for the treatment of PD are lacking. L-Dopa is one of the most commonly used medicines for PD. However, the use of L-dopa can be problematic with regard to the selection of the appropriate dosage and timing. Therefore, it is necessary to identify the optimal timing and dosage of L-dopa and evaluate the efficacy of disease treatment in order to reduce unnecessary drug use.

Positron emission tomography (PET), an imaging technique for use in the *in vitro* quantitation and dynamic observation of physiological and biochemical changes in the body, has been widely applied in aspects of neuro-

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*Correspondence to:* Professor Yi-Wei Wu, School of Radiation Medicine and Protection, Medical College of Soochow University, 199 Ren'ai Road, Suzhou, Jiangsu 215000, P.R. China  
E-mail: wuyiwei3988@gmail.com

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logical diseases. The spatial resolution of PET technology specially designed for use in small animals (micro-PET) is  $\leq 0.8$  mm, which enables dynamic histological analysis to be performed on small volumes (6).  $^{18}\text{F}$ -fallypride, the systematic name of which is 5-(3- $^{18}\text{F}$ -fluoropropyl)-2,3-dimethoxy-N-[(2S)-1-prop-2-enylpyrrolidin-2-yl] methyl] benzamide (structure shown in Fig. 1), is a safe and effective novel imaging agent for DA receptor D2 (DRD2), due to its strong lipophilic property and high affinity for DRD2 in the brain, as well as the short half-life of the carried positron  $^{18}\text{F}$  (~109 min) (7,8). Micro-PET imaging with  $^{18}\text{F}$ -fallypride can be used to observe the changes in brain DRD2 in a live, noninvasive and dynamic manner, and thus provides a novel means for the study of brain diseases (9).

A decline in the levels of DA and its metabolites is the major neurobiochemical change of PD, and it is proportional to the degree of neuronal loss in the substantia nigra (SN). Therefore, the nigrostriatal midbrain DA content is one of the main indicators of clinical diagnosis (10). While the main metabolites of DA include 3,5-dihydroxyphenylacetic acid (DOPAC) and homovanillic acid (HVA), among others, HVA is the final product; therefore, the ratio of HVA/DA can directly reflect the metabolism of DA and the function of residual dopaminergic neurons (11). The immunoreactivity of tyrosine hydroxylase (TH) can also be used as a marker of dopaminergic neurons (12). In the present study a 1-methyl-4-phenyl-1,2,3,6-tetrapyrroline (MPTP)-induced mouse model of PD was established (13), the role of micro-PET imaging in the evaluation of the treatment of PD with L-dopa was verified, and the underlying mechanisms were explored, by methods including observation of general behavior, swimming test, locomotor activity counts, determination of striatal contents of glutathione peroxidase (GSH-PX), superoxide dismutase (SOD), DA, DOPAC and HVA, immunohistochemical analysis of TH and micro-PET imaging.

## Materials and methods

**Reagents and animals.** MPTP was from Atuka Inc. (Toronto, Canada); L-dopa tablet was the product of Beijing Shuguang Pharmaceutical Co., Ltd. (Beijing, China); mouse anti- $\beta$ -actin antibody was purchased from Abcam (Cambridge, MA, USA); GSH-PX, SOD and malondialdehyde (MDA) assay kits were purchased from Nanjing Jiancheng Technology Co., Ltd. (Nanjing, China). Other reagents were of analytical grade. The experimental animals were 63 male 4-week-old ICR mice (20–24 g), provided by Shanghai SLAC Laboratory Animal Co., Ltd. (Shanghai, China; animal certificate no: 2007000522089).

**Synthesis of  $^{18}\text{F}$ -fallypride and determination of the radiochemical purity.** The  $^{18}\text{F}$ -fallypride was prepared by reacting the reaction precursor (4 mg; ABX GmbH, Radeberg, Germany) with resolubilized  $\text{K}[^{18}\text{F}]\text{F-K}_{222}$  (ABX GmbH) in acetonitrile (1 ml) at  $90^\circ\text{C}$  for 20 min. The crude reaction mixture was mixed with water (8 ml) and passed through a Sep-Pak C-18 column chromatography cartridge (Waters Corporation, Milford, MA, USA) 3 times. To determine the radiochemical purity,  $^{18}\text{F}$ -fallypride was loaded onto one end of an instant thin layer chromatography (iTLC) paper and

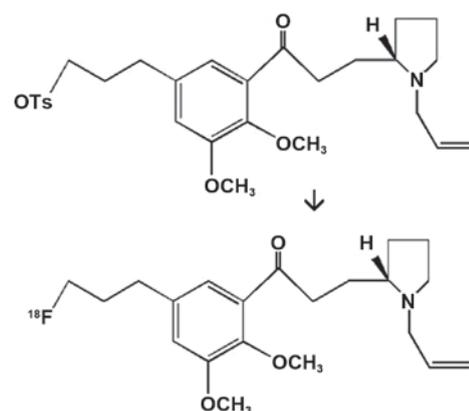


Figure 1. Chemical structure of  $^{18}\text{F}$ -fallypride (lower) and its precursor (upper). OTs, tosylate.

separated upward with dichloromethane:methanol (v/v)=9:1 as the developing agent. The radiochemical purity was measured with a Mini-Scan TLC scanner (Bioscan, Inc., Washington, DC, USA).

## PD mouse model establishment and evaluation

**Establishment of the PD mouse model.** This study was approved by Soochow University review board/local ethics committee (Suzhou, China), and the experiments followed the animal management regulations on experiments in China. A total of 36 ICR mice were intraperitoneally (i.p.) injected with MPTP (25 mg/kg) for 7 consecutive days.

**Assessment of the PD mouse model.** i) General behavioral test. The general behavior of the mice was observed following the injection of MPTP.

ii) Swimming test. According to the method of Donnan *et al* (3), the mice were placed in a Plexiglass tank (20x30x20 cm); the water depth was 10 cm, and the water temperature was  $22\text{--}25^\circ\text{C}$ . The mice were graded as follows: Mice continuously swimming for 1 min received 3.0 points; floating occasionally and swimming most the time scored 2.5 points; floating for >50% of the time received 2.0 points; swimming occasionally received 1.5 points; and mice occasionally swimming with hindlimbs and floating at the side of the water tank were given 1.0 point. An average score was obtained from 3 independent tests with a 10-min interval between each test. The mice were tested once prior to drug administration and again at 1, 4 and 7 days, respectively, after the 40 mg/kg/day treatment, which began at day 7 after modeling.

iii) Locomotor activity counts. According to the method of Kawai *et al* (14), a homemade 30x30x15 cm Plexiglass box was prepared with 6x6 cm grids drawn on the bottom. The test was performed in a quiet, low-light environment. The mice were adapted to the environment for 10 min and then the number of lines crossed and the frequency of standing posture were determined. An average value was obtained from 3 independent tests with a 20-min interval between each test. The mice were tested once prior to the drug administration and again at 1, 4 and 7 days, respectively, after the treatment.

## $^{18}\text{F}$ -fallypride imaging and competitive inhibition experiments

*Micro-PET imaging in vivo with <sup>18</sup>F-fallypride.* Following anesthesia with ketamine, the mice were placed onto the headboard of micro-PET (Inveon micro-PET; Siemens AG, Munich, Germany) and injected with <sup>18</sup>F-fallypride (3.7 MBq/each) through the tail vein. The acquisition mode was as follows: Energy peak, 511 keV; time window, 3.432 nsec; acquisition time, 120 min. The attenuation-corrected images of <sup>18</sup>F-fallypride distribution *in vivo* in the mice were obtained using an iterative reconstruction method. Region of interest (ROI) techniques were used to manually select the striatal ROI in the coronal section of mice and calculate the maximum uptake of <sup>18</sup>F-fallypride in the ROI.

*Competitive inhibition binding experiment with <sup>19</sup>F-fallypride.* Appropriate amounts of <sup>19</sup>F-fallypride were weighed and used to prepare standard solutions at the concentrations of 500, 50, 10, 1 and 0.1  $\mu$ g/ml, respectively. Eighteen normal ICR mice were randomly divided into 6 groups with 3 animals in each group, which were injected with respective <sup>19</sup>F-fallypride standard solutions (100  $\mu$ l/20 g body weight) via tail vein. Mice injected with an equal volume of saline served as the negative control group. Low to high concentrations of <sup>19</sup>F-fallypride were sequentially injected into each group; 10 min later, the <sup>18</sup>F-fallypride was injected, and after another 10 min, the animals were scanned by micro-PET. ROIs were then used to outline the striatal radioactive counts.

*Determination of TH level by immunohistochemistry.* The mice were weighed, anesthetized with ketamine and perfused with 4% paraformaldehyde. The brains were dissected immediately after the perfusion, fixed in 4% paraformaldehyde for 2 h and placed sequentially into 20 and 30% sucrose solutions. After the tissues sank to the bottom, the SNpc and caudate nucleus were selected and cut into serial coronal frozen sections (thickness, 20  $\mu$ m). The sections were then washed 3 times in phosphate-buffered saline (PBS; 0.01 M) by shaking for 15 min each time, incubated with 0.3% H<sub>2</sub>O<sub>2</sub> for 10 min at room temperature to deactivate the endogenous peroxidase activity, and blocked with PBS containing 10% normal goat serum and 0.2% Triton X-100. The sections were incubated with mouse monoclonal anti-TH antibody (1:1,000; cat. no. ab49640; Abcam) for 24 h. The immunoreactivity of TH in the SN was observed under a microscope (CX21; Olympus Corporation, Tokyo, Japan) at low magnification to calculate the number of neurons in the mouse brain structure.

*Determination of the contents of GSH-PX, SOD and MDA.* The mice were divided into control, model and L-dopa 1 day, 4 day and 7 day treatment groups with 6 animals in each group. Following anesthesia by the intraperitoneal injection of 10% chloral hydrate, the mice were decapitated, and the whole brain was rapidly removed and placed on an ice-cold tray. The striatum was then separated, weighed and homogenized. The homogenate was adjusted to a concentration of 10% and stored at -80°C. The contents of GSH-PX, SOD and MDA in the striatal homogenates were measured according to the kit instructions.

*Transmission electron microscopy (TEM).* The brains of 3 mice per group were fixed by perfusion with 4% paraformaldehyde and 2% glutaraldehyde; the SN was then isolated,

Table I. Striatal uptake of <sup>18</sup>F-fallypride under different concentrations of <sup>19</sup>F-fallypride (n=3).

Concentration ( $\mu$ g/ml)	Uptake (% ID/g)
Blank	5.98±0.48
500	0.48±0.04
50	1.03±0.12
10	3.32±0.29
1	4.04±0.17
0.1	4.58±0.31

ID, injected dose.

treated with osmium tetroxide, dehydrated, and embedded in epoxy resin. Frozen sections (90 nm) were cut and observed under a Hitachi H600 TEM (70 kV; Hitachi, Tokyo, Japan).

*Determination of the striatal content of DA and its metabolites by high performance liquid chromatography-electrochemical detection (HPLC-ECD).* The brains of mice from each group were immediately dissected following decapitation of the animal to isolate the striatum, which was preserved in liquid nitrogen prior to testing. The striatum was homogenized in 200  $\mu$ l 0.02 M perchloric acid and centrifuged at 4°C, 12,000 x g for 30 min to remove proteins. The content of DA and its metabolites DOPAC and HVA in the supernatant were detected using the HPLC-ECD method under the following conditions: Mobile phase, 0.1 M NaH<sub>2</sub>PO<sub>4</sub> buffer (pH 3.25) containing 0.85 M sodium 1-octanesulfonate, 11% methanol and 0.5 mM EDTA-Na<sub>2</sub>; applied potential, 0-500 mV at an increment of 100 mV; column temperature, 35°C; flow rate, 1.2 ml/min, and injection volume, 50  $\mu$ l. The DA content was represented in units of  $\mu$ g/g wet tissue weight.

*Statistical analysis.* All data were analyzed for single-factor analysis of variance using SPSS software, version 18.0 (SPSS, Inc., Chicago, IL, USA). The results were presented as mean  $\pm$  standard deviation. Comparison between two groups was conducted by 2-sample t-test. P<0.05 was considered to indicate a statistically significant result.

## Results

*Labeling yield and radiochemical purity of <sup>18</sup>F-fallypride and competitive inhibition results with <sup>19</sup>F-fallypride.* Table I and Fig. 2 show the uptake of <sup>18</sup>F-fallypride in the striatal area 10 min after the injection and the dose of <sup>19</sup>F-fallypride. The uptake of <sup>18</sup>F-fallypride increased significantly with the lowering of the <sup>19</sup>F-fallypride dosage (Fig. 2).

*Changes of the evaluation indices in the mouse model of PD*  
*General behavioral test.* The behavioral changes of the mice in the PD model group were mainly as follows: Slow movement, arched back, hair erection, tail stiffness, increased salivation, rapid breathing and trembling head and teeth, which might last for up to 2-3 h. The behavioral changes in

Table II. Results of the swimming test prior to treatment and following 1, 4 and 7 days of treatment with L-DA (n=6).

Groups	Test score			
	Prior to treatment	Day 1	Day 4	Day 7
Control	2.81±0.13	2.83±0.18	2.81±0.16	2.81±0.19
Model	2.38±0.25 <sup>a</sup>	2.46±0.08 <sup>a</sup>	2.50±0.24 <sup>b</sup>	2.46±0.21 <sup>b</sup>
L-DA	2.44±0.25 <sup>b</sup>	2.52±0.17 <sup>b</sup>	2.63±0.21	2.75±0.17

Results are presented as mean ± standard deviation. <sup>a</sup>P<0.01, <sup>b</sup>P<0.05 vs. the control group. L-DA, L-dopa.

Table III. Results of locomotor activity prior to treatment and following 1, 4 and 7 days of treatment with L-DA (n=6).

Groups	No. of lines crossed			
	Prior to treatment	1 day	4 days	7 days
Control	137.13±22.53	142.11±23.75	140.33±16.11	135.43±21.07
Model	63.19±18.43 <sup>a</sup>	67.34±6.53 <sup>a</sup>	75.25±24.65 <sup>a</sup>	71.13±33.42 <sup>b</sup>
L-DA	60.43±23.53 <sup>a</sup>	88.52±30.21 <sup>b</sup>	111.95±11.07 <sup>c,d</sup>	129.64±24.53 <sup>d</sup>

Results are presented as mean ± standard deviation. <sup>a</sup>P<0.001, <sup>b</sup>P<0.01, <sup>c</sup>P<0.05 vs. the control group; <sup>d</sup>P<0.05 vs. the PD model. L-DA, L-dopa, PD, Parkinson's disease.

Table IV. Frequency of standing posture of mice in each group (times/5 min) (n=6).

Groups	Frequency of standing posture			
	Prior to treatment	1 day	4 days	7 days
Control	38.03±5.39	39.15±5.53	39.01±5.04	38.30±4.53
Model	24.25±6.34 <sup>a</sup>	23.53±4.31 <sup>a</sup>	27.53±6.53 <sup>b</sup>	31.25±5.35
L-DA	21.05±7.91 <sup>c</sup>	32.15±4.53 <sup>b,d</sup>	35.05±7.35	36.13±7.25

Results are presented as mean ± standard deviation. <sup>a</sup>P<0.01, <sup>b</sup>P<0.05, <sup>c</sup>P<0.001 vs. the control group; <sup>d</sup>P<0.05 vs. the PD model group. L-DA, L-dopa, PD, Parkinson's disease.

the L-dopa-treated group were mild and with shorter duration.

**Swimming test.** The results of the swimming test for mice in the control, PD model and L-DA groups are shown in Table II. The swimming times of mice in the model group prior to drug treatment were significantly higher than those in the control group. After 4 days of L-dopa administration, while the swimming times in the model group remained shorter than those in the control group, the swimming times in the treatment group showed no difference compared with those in the control group.

**Mouse locomotor activity.** The line-crossing and frequency of standing posture results of mice in each group are shown in Tables III and IV. Prior to drug administration, the number of lines crossed and the times of standing of mice in the model group were significantly less than those in the control group; however, after 7 days of L-dopa treatment, the number of lines crossed and the times of standing in the

treatment group showed no significant difference from those in the control group.

**Neuronal changes in the SNpc.** Fig. 3 shows the morphological changes of neurons in the SNpc of mice from each group. The neurons in the SNpc of the control mice were easily observed, and their nuclei contained a large circular cluster of chromatin with clear boundaries; a large amount of complete mitochondria and endoplasmic reticulum were also observed in the cytoplasm. However, morphological changes occurred in the cytoplasm and nuclei of neurons in the SNpc in the PD model group. The electron density in the nuclei increased and swelling of the perinuclear region was observed. Cytoplasmic vacuoles and a multilayer structure with alternating ribosomes and expanded endoplasmic reticulum were also observed. L-dopa treatment attenuated the neuronal damage to a certain extent. With prolonged drug administration, the number of cytoplasmic vacuoles

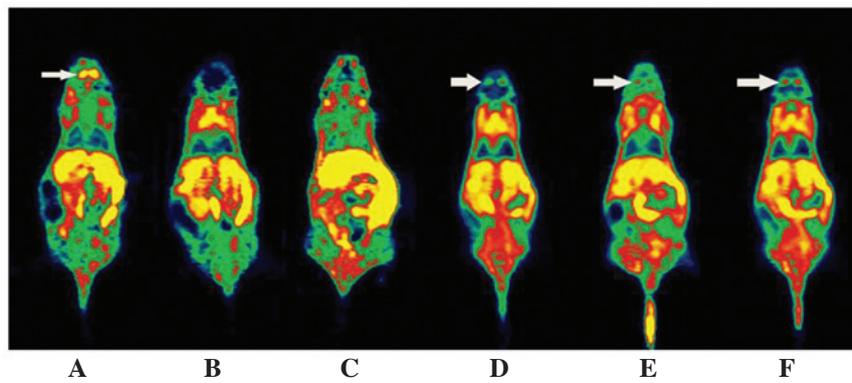


Figure 2. Striatal uptake of  $^{18}\text{F}$ -fallypride after the injection of (A) 0, (B) 500, (C) 50, (D) 10, (E) 1 and (F) 0.1  $\mu\text{g/ml}$   $^{19}\text{F}$ -fallypride. Arrow indicates striatum.

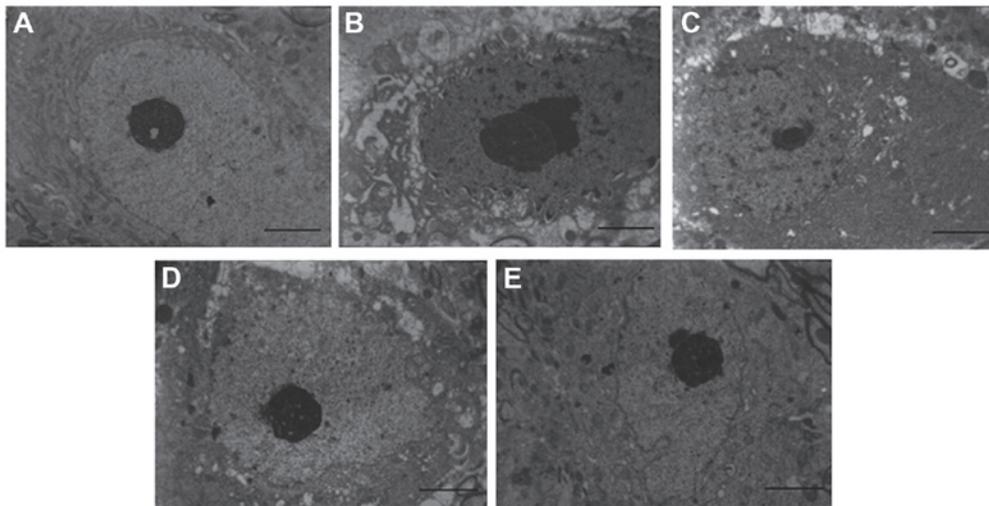


Figure 3. Transmission electron microscopy images of neuronal changes in mouse SNpc. (A) Ultrastructure of a normal mouse neuron and neuronal morphology in (B) the PD model group and in mice treated with L-dopa for (C) 1, (D) 4 and (E) 7 days. Scale bar, 2  $\mu\text{m}$ . SNpc, substantia nigra pars compacta; PD, Parkinson's disease.

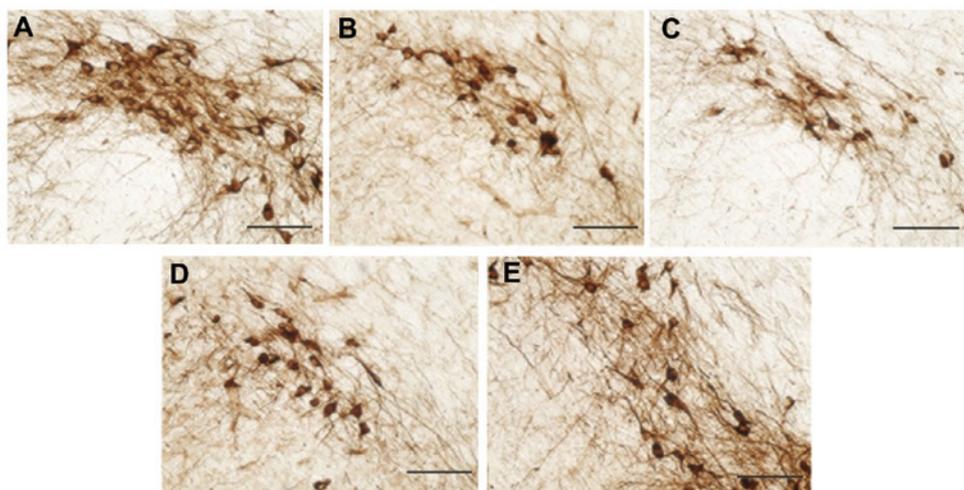


Figure 4. Microscopic images of TH immunoreactivity in the SNpc of mice from each group. (A) Control group; (B) PD model group; mice treated with L-dopa for (C) 1, (D) 4 and (E) 7 days, respectively. Scale bar, 100  $\mu\text{m}$ . TH, tyrosine hydroxylase; SNpc, substantia nigra pars compacta; PD, Parkinson's disease.

was reduced, and mitochondria of normal size and shape appeared.

*TH immunohistochemistry.* Images of TH immunostaining in the mice, captured under a microscope, are shown in

Table V. Striatal levels of MDA, SOD and GSH-PX in mice of each group (n=6).

Groups	MDA (nmol/mg protein)	SOD (U/mg protein)	GSH-PX (U/mg protein)
Control	8.65±0.35	4.23±0.16	135.13±21.45
Model	17.12±0.56 <sup>a</sup>	2.36±0.27 <sup>a</sup>	49.24±4.63 <sup>a</sup>
L-Dopa 1 day	15.43±0.95	2.76±0.54	58.42±8.53
L-Dopa 4 day	10.57±1.25 <sup>b</sup>	3.15±0.89 <sup>b</sup>	84.25±8.03 <sup>b</sup>
L-Dopa 7 day	7.53±0.43 <sup>c</sup>	4.54±0.91 <sup>b</sup>	114.54±7.34 <sup>c</sup>

Results are presented as mean ± standard deviation. <sup>a</sup>P<0.05 vs. the control group; <sup>b</sup>P<0.05, <sup>c</sup>P<0.01 vs. the PD model. MDA, malondialdehyde; SOD, superoxide dismutase; GSH-PX, glutathione peroxidase; PD, Parkinson's disease.

Table VI. Results of the micro-PET imaging of mice in each group (n=6).

Groups	SUV (% ID/g)			
	Prior to treatment	1 day	4 days	7 days
Control	3.57±0.10	3.65±0.38	3.62±0.60	3.74±0.15
Model	2.84±0.22 <sup>a</sup>	2.71±0.13 <sup>b</sup>	2.97±0.27	3.00±0.14 <sup>a</sup>
L-DA	2.77±0.11 <sup>a</sup>	3.17±0.11 <sup>c</sup>	3.41±0.08 <sup>d</sup>	3.42±0.15 <sup>c</sup>

<sup>a</sup>P<0.001, <sup>b</sup>P<0.01 vs. the control group; <sup>c</sup>P<0.001, <sup>d</sup>P<0.05, <sup>e</sup>P<0.01 vs. the PD model group. PET, positron emission tomography; SUV, standardized uptake value; ID, injected dose; PD, Parkinson's disease; L-DA, L-dopa.

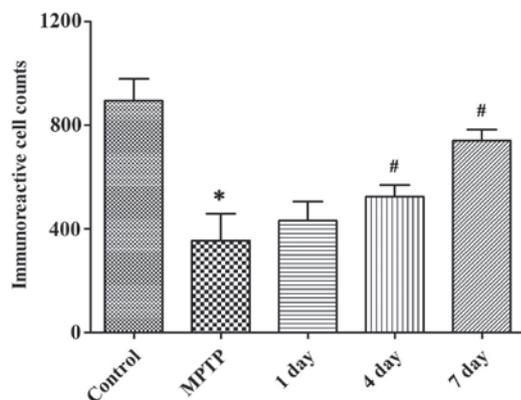


Figure 5. Number of TH-positive neurons in the SNpc of each group. <sup>\*</sup>P<0.05 vs. the control group; <sup>#</sup>P<0.05 vs. the PD model (MPTP) group. TH, tyrosine hydroxylase; SNpc, substantia nigra pars compacta; PD, Parkinson's disease; MPTP, 1-methyl-4-phenyl-1,2,3,6-tetrapyridine.

Fig. 4, while Fig. 5 shows the number of TH-positive neurons. Compared with the control group, mice in the PD (MPTP) model group showed a significantly reduced number of TH-positive neurons, the survival rate of which was only 34.66%. However, the number of TH-positive neurons in the L-dopa-treated group was significantly increased (P<0.05).

**MDA, SOD and GSH-PX levels in the mouse striatum.** The striatal contents of MDA, SOD and GSH-PX are shown in Table V. The striatal MDA content in the PD model group increased significantly compared with that in the control

group, (P<0.05); however, the GSH-PX and SOD contents were significantly lower in the model group than in the control group (P<0.05). Compared with the PD model group, the L-dopa-treated group displayed significantly increased levels of GSH-PX and SOD, and a reduced content of MDA (P<0.05).

**Effect of L-dopa on the striatal contents of DA and its metabolites in mice.** As shown in Fig. 6, compared with the control group, mice in the PD model group showed significantly declined striatal contents of DA, DOPAC and HVA (P<0.05). In comparison with the PD model group, the striatal contents of DA were significantly increased after 4 days of L-dopa treatment (P<0.05). In addition, the DOPAC/DA ratio in the PD model group was significantly higher than that in the control group, and L-dopa treatment strongly inhibited the effect of MPTP-induced monoamine oxidase (MAO)-dependent DA metabolism (P<0.05) on DOPAC/DA and HVA/DA ratios. Similarly, compared with the control group, the total DA metabolic rate HVA/DA in the PD model group increased significantly, but L-dopa administration significantly reduced the DA metabolic rate (P<0.05).

**Micro-PET imaging.** The results of micro-PET scanning of mice in each group at different time-points are shown in Table VI and Figs. 7 and 8. The striatal uptake in the model and treatment groups was significantly lower than that in the control group prior to the drug administration (P<0.01). After 1 and 7 days of drug treatment, while the striatal uptake in the model group was lower than that in control group, the

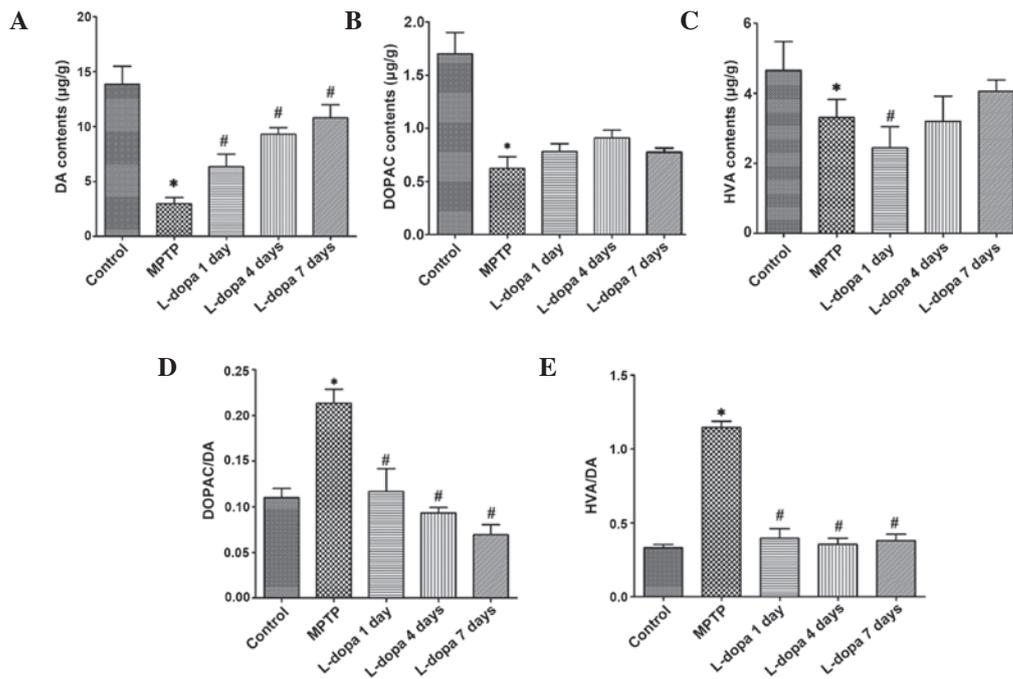


Figure 6. Effect of L-dopa on the levels of DA and its metabolites. Levels of (A) DA, (B) DOPAC and (C) HVA, and the (D) DOPAC/DA and (E) HVA/DA ratios in each group. \*P<0.05 vs. the control group; #P<0.05 vs. the PD model (MPTP) group. DA, dopamine; DOPAC, 3,5-dihydroxyphenylacetic acid; HVA, homovanillic acid; PD, Parkinson's disease; MPTP, 1-methyl-4-phenyl-1,2,3,6-tetrapyridine.

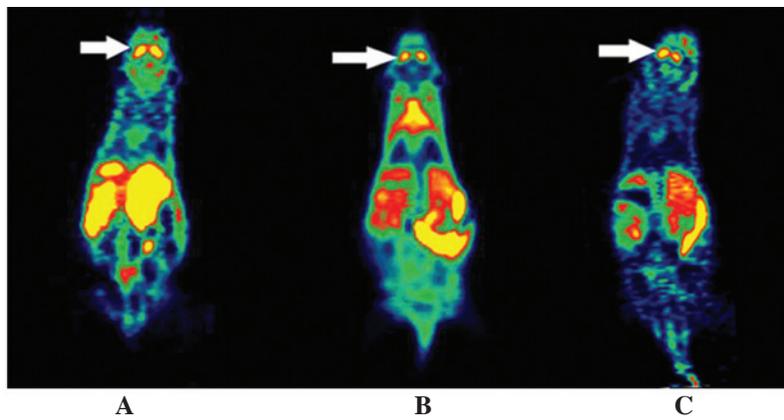


Figure 7. Imaging results of mice in each group prior to L-dopa administration. (A) Control, (B) model and (C) L-dopa treatment groups. Arrow indicates striatum.

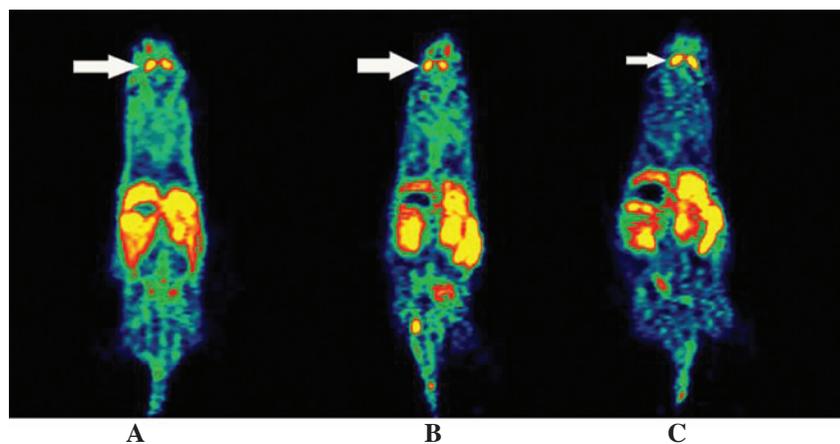


Figure 8. Imaging results of mice in each treatment group 7 days after L-dopa administration. (A) Control, (B) model and (C) L-dopa treatment groups. Arrow indicates striatum.

L-dopa intervention group showed an evidently increased striatal uptake compared with model group, which was not significantly different from that in the control group.

## Discussion

The method for <sup>18</sup>F-fallypride synthesis is simple, with high product stability and synthesis efficiency. The radiochemical purity of the obtained <sup>18</sup>F-fallypride was >96%. In this study, the <sup>19</sup>F-fallypride competitive inhibition test showed that the uptake of <sup>18</sup>F-fallypride increased as the <sup>19</sup>F-fallypride dose decreased, which is consistent with the results of micro-PET imaging, indicating that <sup>18</sup>F-fallypride is a specific ligand of the DA receptor and may be used to reflect the expression of the DA receptor (15).

An MPTP-induced mouse model of PD was used in the present study. After MPTP injection, the number of TH-positive striatal neurons reduced significantly; the behavioral characteristics manifested as reduced activities and slow movement. TEM revealed morphological changes in the nuclei and cytoplasm of neurons in the SNpc, a reduced number of cytoplasmic vacuoles, and abnormalities in mitochondrial size and morphology in the PD model group. Micro-PET also showed that compared with the control group, mice in the PD model group had a significantly declined uptake of <sup>18</sup>F-fallypride, which is consistent with the characteristic pathological changes of PD patients and previous findings (16,17).

The present study also investigated the effects of L-dopa treatment on PD model mice, and the results of behavioral experiments showed that L-dopa alleviated the symptoms of PD to a certain extent. TEM also demonstrated that L-dopa administration reduced the MPTP-induced morphological changes in the nuclei and cytoplasm of neurons in the SNpc; for example, it reduced the number of cytoplasmic vacuoles and normalized the mitochondrial size and morphology. However, treatment of the PD mice with L-dopa significantly increased the number of TH-positive cells, and compared with the PD model group, L-dopa administration significantly reduced the levels of DOPAC/DA and HVA/DA, these ratios were significantly recovered to a level close to that of the control group, indicating that L-dopa treatment attenuated the metabolic rate of DA and thereby improved the behavioral disorders in the PD mice (12).

Previous studies have determined the effect of PD therapy only through behavioral methods and clinical characteristics, and there is lack of a scientific research aimed at identifying a simple and easy noninvasive method of examination. The present study observed clear images of <sup>18</sup>F-fallypride by micro-PET imaging, which showed that the striatal uptake of <sup>18</sup>F-fallypride in the L-dopa treatment group significantly increased compared with that in the PD model group on day 1 of treatment ( $P < 0.05$ ), and had no significant difference from that in control group 7 days after the treatment ( $P > 0.05$ ). It was also found that the standardized uptake value (SUV) of <sup>18</sup>F-fallypride in the micro-PET imaging results was clear and reliable, which forms a valuable reference for monitoring the efficacy of PD therapy in a mouse model and provides a new means of observation (18).

Studies suggest that the reduction in the number of TH-positive cells and DA receptor levels in the mice model

of PD reproduces the clinicopathological features of PD patients (19,20). L-dopa alleviated the symptoms of mice with PD by upregulating the number of TH-positive cells and the level of the DA receptor as well as attenuating the metabolic rate of DA. These changes may be observed noninvasively *in vitro* by <sup>18</sup>F-fallypride imaging, a new method for monitoring the early efficacy of PD treatment. However, few current clinical studies have focused on <sup>18</sup>F-fallypride (21). Therefore, whether PET imaging can be used for the early diagnosis of early-stage PD requires further verification by clinical imaging studies of patients with PD. Furthermore, whether <sup>18</sup>F-fallypride imaging can be used as a means to monitor the therapeutic efficacy and evaluate the prognosis of PD also requires further confirmation through investigations with drug intervention and PET imaging in patients with PD.

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