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Research report

Effects of D-cycloserine on MPTP-induced behavioral and neurological changes: Potential for treatment of Parkinson's disease dementia

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ABSTRACT

Glutamatergic dysfunction has been implicated in the neurodegeneration seen in Parkinson's disease (PD). Sub-chronic intraperitoneal injection with D-cycloserine (DCS), a partial agonist at the glycine binding site of the N-methyl-D-aspartate (NMDA) receptor, at dosages of 30, 100, or 200 mg/kg/day, was used to evaluate the role of NMDA receptors in neuronal and behavioral changes in a 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP)-induced PD rat model. Starting one day after intra-nigral infusion of MPTP, transient disturbance of motor function in the rotarod test was observed. This impairment spontaneously recovered to control levels 6 days after MPTP lesioning and DCS treatment facilitated recovery. MPTP lesioning also caused deficits in working memory and anxiety-like behavior in the T-maze and elevated plus-maze tests, respectively. Further, object recognition was disrupted in MPTP-lesioned rats, and interleukin-2 levels in the striatum, amygdala, and non-prefrontal cortex were increased, both changes being restored by DCS treatment. Furthermore, MPTP lesion-induced dopaminergic degeneration, microglial activation, and cell loss in the hippocampal CA1 area were all improved by DCS treatment. These results suggest that NMDA receptors are involved in PD-related neuronal and behavioral dysfunctions and that DCS may have clinical potential in the treatment of dementia associated with PD.

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1. Introduction

In addition to motor dysfunction, cognitive impairment and dementia are seen in a high percentage of patients with Parkinson's disease (PD) [12,69]. The proportion of PD patients with dementia is around 25–30% [1]. Emotional changes [53], psychotic disorders [22], executive dysfunction [22], and working memory and visuospatial impairments [16,22] are the main symptoms in Parkinson's disease dementia (PDD). However, the development of drug therapy for dementia in PD has been hampered because the pathophysiology is not yet fully understood.

Physiologically, PD is mainly characterized by the loss of dopaminergic (DAergic) neurons in the substantia nigra [59]. Neuroinflammation and neurodegeneration have been proposed to be involved in the pathogenesis of PD [2,59]. Increased levels of inflammatory cytokines have also been found in the nigrostriatal regions and in the cerebrospinal fluid (CSF) in PD patients

[63,64,66]. Clinical studies have shown that interleukin (IL)-2 levels are increased in the caudate nucleus [61] and CSF [62] in patients with PD, and this increase correlates with psychiatric disorders [55,58]. Since DAergic neurons are particularly vulnerable to inflammatory cytokines [59], cytokines have been implicated in the neuronal and cognitive impairments in PD [44]. 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP), a neurotoxin that selectively damages DAergic cells in the substantia nigra pars compacta (SNc), is widely used to induce models of PD in rodents and primates [18,25,26,60,84]. MPTP causes not only motor dysfunction and DAergic degeneration in the brain [13,25], but also neuroinflammation [45], for example, inducing microglial activation [92,96] and increasing levels of inflammatory cytokines, including IL-2, in animals [98] and humans [62]. Activated microglia can release IL-2, which might be involved in the neuroinflammation [104]. Thus, activated microglia and IL-2 levels may be good markers for monitoring neuronal changes in animal models of PD. Further, hyperactivity of the glutamatergic system, seen as a transient increase in glutamate efflux in the brain [82], has also been observed after MPTP lesioning. When glutamate binds, together with glycine, to N-methyl-D-aspartate (NMDA) receptors, it can cause opening of the channel and calcium influx, leading

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to neuronal excitation. Thus, excessive glutamate release in the brain has been implicated in excitotoxic neuronal death following MPTP lesioning [15,77,79]. Impairment of executive and visuospatial functions is observed not only in patients with PDD [16,22], but also in people exposed to MPTP [91]. In addition, cognitive impairment has been observed in MPTP-lesioned rats in the two-way active avoidance task [26] or Morris water maze test [18,25,60], as well as in monkeys in object recognition tasks [84]. Our previous study demonstrated that the behavioral deficits observed in MPTP-lesioned rats may be similar to the behavioral symptoms seen in PDD patients [92,98]. The above data suggest that dysfunction of glutamatergic activity may be involved in the neuroinflammation, neurodegeneration, and cognitive deficits in PD. Thus, drugs modulating the function of NMDA receptors may have beneficial effects in PDD therapy.

NMDA receptors in the hippocampus mediate not only learning and memory [88], but also object recognition [31]. D-cycloserine (DCS), a partial agonist of the glycine binding site of the NMDA receptor, improves spatial navigation and learning deficits in aged rats [6,7,81], anxiety-like behavior in rats [33,100], and object recognition in MPTP-lesioned monkeys [84]. DCS can also improve brain damage-induced impairment of long-term potentiation (LTP) in the hippocampus [103] and restore impairments in neurodegeneration and episodic-like memory in MPTP-lesioned rats [96]. Intra-hippocampal injection of DCS has been reported to reverse MK-801-induced memory deficits in rats [43]. However, it is not known whether DCS treatment affects MPTP-induced emotional and cognitive deficits in rats. In order to further evaluate the potential of DCS in PDD therapy, we examined its effects on motor behavior, working memory, emotional behavior, and object recognition in rats after MPTP lesioning using a battery of behavioral tests. In addition, we analyzed IL-2 levels and neurohistological changes in the brain. Our results showed that DCS treatment improved MPTP-induced behavioral and neurological deficits. We therefore suggest that DCS may have beneficial effects in PDD therapy.

2. Materials and methods

2.1. Animals

Male Wistar rats (415.9 ± 4.0g; National Laboratory Animal Center, ROC) were housed in groups of five in acrylic cages (35 cm × 56 cm × 19 cm) in an animal room with a 12 h light–dark cycle (lights on at 07:00h) with food and water available *ad libitum*. Each animal was handled for 5 min/day on 3 consecutive days, starting one day after arrival. All experimental procedures were performed according to the NIH Guide for the Care and Use of Laboratory Animals and were approved by the Animal Care Committee of Chung Shan Medical University (IACUC approval no.: 434).

2.2. General procedure

All animals underwent stereotaxic surgery and bilateral infusion of MPTP–HCl (1 μmol in 2 μl of saline; Sigma, MO, USA) or vehicle on day 0 into the substantia nigra pars compacta (SNc) (see surgery section below), as in previous reports [17,26,92,96,98]. One day after surgery, the rats received daily intraperitoneal (i.p.) injections of DCS (30, 100, or 200 mg/kg/day; Sigma, USA) or saline in a volume of 1 ml/kg ($n = 21–22$ per group) at 18:00h for 13 days. The rationale of using these dosages in the present study was based on our previous studies, where the treatment of DCS at the dosages of 10 or 30 mg/kg caused behavioral changes in the elevated plus-maze [100] and episodic-like memory [96] tests. For analyzing whether higher dose of DCS has higher or even reversed effect, two higher doses were applied in this study. The rats were subjected to a battery of behavioral tests performed as in our previous studies [92,98]: the rotarod test was performed on days 1–7, the T-maze test on days 8–10, the elevated plus-maze test on day 11, and the object recognition test was started on day 11 and finished on day 13. All behavioral observations were started at least 2 h after the beginning of the light phase (7:00h). For behavioral testing, the animals were weighed in the animal room, placed individually in a clean cage (25 cm × 41 cm × 19 cm), and transported to a dim observation room (28 lx) with sound isolation reinforced by a masking white noise of 70 db. Performance in the behavioral tests was monitored using a video camera positioned above the apparatus and a home-made video image analysis system (VIAS) [54]. Data were acquired and scored using the VIAS and in-house-developed software. The spatial

resolution of the VIAS was set to 0.7 cm and the image processing capability was higher than 14 pictures/sec. Some of the animals were randomly assigned to the rotarod test ($n = 11–12$ for each group) or T-maze test ($n = 11–12$ for each group), and the behavior in these two tests was scored manually by a trained observer blind to the treatment conditions. The test equipment and objects used in this study were cleaned using 20% ethanol and thoroughly dried before each test trial. On day 14 after MPTP lesioning, the rats were euthanized by exposure to CO₂ from gas canister, transcardially perfused with phosphate-buffered saline, and the brain immediately removed for histological examination and IL-2 assay.

2.3. Surgery

Brain surgery was performed as in our previous report [92,96,98]. Briefly, the rats were anesthetized using Zoletil (20 mg/kg, i.p.; Virbac, Carros, France), then MPTP–HCl (1 μmol in 2 μl of saline) was bilaterally infused into the SNc with the following coordinates adapted from the rat brain atlas [72]: AP: –5.0 mm, ML: ±2.0 mm, DV: –8.0 mm from the bregma, midline, and skull surface, respectively. Controls were subjected to the same procedure, but were infused with 2 μl of saline instead of MPTP. Immediately after surgery, the rats were injected with penicillin-G procaine (0.2 ml, 20,000 IU, IM) and housed individually in plastic cages (25 cm × 41 cm × 19 cm) for a week, then they were re-grouped in their home cages (rats from the same home cage underwent the same treatment). During the first 5 post-operative days, 10% sucrose solution was provided *ad libitum* to prevent weight loss after surgery and reduce mortality [17,25].

2.4. Behavioral tests

2.4.1. Rotarod test ($n = 11–12$ for each group)

Motor function was assessed using an automated rotarod (Rotarod, RT-02, Singa, Taiwan), as described previously [95]. Briefly, the rats were trained daily on the rotarod (constant speed of 18 rpm; rod diameter 9 cm) for 3 consecutive days before MPTP lesioning, at which time the rats were able to keep walking on the rod for at least 30 s (taken as the maximum time). Starting one day after the MPTP lesion, the rats were tested daily on the rotarod for 7 consecutive days. Each test included 3 trials (maximum of 30 s per trial) with a 30 s inter-trial interval. Latency to falling off the rod was recorded and the data are presented as the percentage of the maximum walking time (30 s) on the rotarod.

2.4.2. T-maze test ($n = 11–12$ for each group)

The construction of the T-maze and the test procedures were similar to those described previously [4]. Briefly, the T-maze was constructed of black polyvinyl plastic. A stem alley (60 cm long × 15 cm wide × 30 cm high) was connected to an arm alley (95 cm long × 10 cm wide × 30 cm high) in a T shape. At the entrance of each arm, there was a sliding door that could be closed, thus forcing rats to run into the other arm. The training session consisted of 9 trials, each composed of two parts. The first part was the forced run in which one of the arms, left or right according to a random order, was closed by a sliding door and the rat was put into the stem alley and allowed to explore the maze, i.e. the stem and open arm. A food cup containing chocolate balls (Kellogg's, Taiwan) was located at the end of the arm as a reward. After entering the arm and getting the reward, the rat was removed and put in a cage adjacent to the T-maze. The second part was the choice run, carried out 30 s later, when the rat was again allowed to run in the maze, but with both arms open. Choosing the newly opened arm, the opposite to that used in the forced run, was the correct response to get the reward. The forced run and choice run were performed one by one for 9 times for each rat in 2 consecutive training days, then, on the following test day, 3 forced-choice-choice run trials were carried out, where rats made 2 choices following a single forced run, and correct responses in the 6 choice runs were recorded. On the day before T-maze training, the rats were partially food restricted, the diet only being provided for 1 h, while, on the 2 training days, the diet was provided for only 1 h after the behavioral observation on that day.

2.4.3. Elevated plus-maze test ($n = 20–22$ for each group)

The construction of the elevated plus-maze and the testing procedures were identical to those in our previous report [33]. The following three measurements were recorded: (1) arm time: the time spent in the open and enclosed arms, (2) enclosed arm activity: the number of times the animal crossed a virtual line dividing an arm into a proximal and a distal half, and (3) rearing number: the number of times the animal reared up on the hind legs during the test. Entry into any of the compartments was defined by the center of the body being placed in the compartment.

2.4.4. Object recognition test ($n = 21–22$ for each group)

The apparatus, an open box (100 cm long × 100 cm wide × 60 cm high), and the test procedure for the object recognition test were identical to those in our previous reports [92,98]. Each rat was subjected to 3 exposure sessions at 24 h intervals, then, 5 min after the last exposure session, a test session was performed. Four different objects made of transparent glass, paper, porcelain, or metal (all about 10 cm × 10 cm × 10 cm) were used for each rat. All objects were unfamiliar to the rats before the experiment. Three of the objects ("A", "B", and "C") were fixed to the floor 27 cm from three corners of the arena. Starting on day 11 after MPTP lesioning

(5 min after the elevated plus-maze test), the rat was allowed to explore the objects for 5 min in the open box on 3 consecutive days, then, 5 min after the last exposure session, object "B" was replaced by a novel object "D" and the animal was returned to the open box for a 5 min test session. The time spent exploring the objects and the number of rearings during the exposure and test sessions were recorded. Exploration of an object was defined as the rat approaching it and making physical contact with it with its snout and/or forepaws. The percentage of exploration time spent on object B or D in the session $[(\text{Time}_B \text{ or } D / \text{Time}_{\text{all objects}}) \times 100\%]$ was calculated. The difference of percentage of time spent exploring object "B" in exposure 3 and on the novel object "D" served as a measure of recognition memory for the familiar object.

2.5. Measurement of IL-2 levels

The prefrontal cortex, non-prefrontal cortex (the cortex tissue excluding the prefrontal cortex), amygdala, striatum (ventral and dorsal part), and hippocampus were dissected on an ice-cold plate and stored at -80°C until use ($n = 12-14$ for each group). The procedures for IL-2 measurement were identical to those used in our previous reports [34,98]. Briefly, IL-2 levels in a sample containing about 30–40 μg of total protein were measured using an enzyme linked immunosorbent assay (ELISA) kit using monoclonal anti-rat IL-2 antibody (CytoSets™, BioSource, CA, USA) according to the manufacturer's instructions.

2.6. Histological assay and image analysis

For histological assessment, 4 randomly selected rats per group were perfused intracardially with 4% paraformaldehyde in phosphate-buffered saline, then the brains were rapidly removed and post-fixed in 20% sucrose solution containing 4% paraformaldehyde at 4°C until use. To detect DAergic degeneration and microglial activation, frozen coronal brain sections ($30\ \mu\text{m}$) were cut and immunostained at 4°C overnight with mouse monoclonal antibodies against rat tyrosine hydroxylase (TH) (1:2000; Zymade, USA) or rat MHC class II (OX-6; 1:200; BD Biosciences Pharmingen, CA, USA), a method identical to that used in our previous reports [92,96,98]. In sections containing the hippocampus, Nissl staining was used to identify neurons.

The stained brain sections, identified according to the rat brain atlas [72], were used to measure histological changes as described previously [98,102] using a microscope (ZEISS AXioskop2, Germany) coupled to a CCD (Optronics, USA) and Image Pro Plus Software 6.0 (Media Cybernetics, CA, USA). In this study, we created three square areas of interest, one $36,477\ \mu\text{m}^2$ in the striatum to determine the optical density of TH immunoreactivity, and one $18,769\ \mu\text{m}^2$ in the SNc, and another $2,354\ \mu\text{m}^2$ in the hippocampal CA1 area to determine neuronal density in these regions. To measure the intensity of DAergic projections in the striatum, we converted the TH-stained images to gray-scale, then measured the gray level of a given area of interest, and subtracted the background staining, measured in the non-immunoreactive corpus callosum. Thus, the relative optical density was restricted to the values generated by the TH-reactive tissue. To measure the density of DAergic neurons in the SNc, images were captured, but not converted to gray-scale, and an area of interest was overlaid in this region and the somas of TH-immunoreactive neurons located in this area counted. The density of activated microglia in the SNc and striatum was measured in the areas of interest (measuring $18,769$ and $36,477\ \mu\text{m}^2$, respectively). Because the neurons were tightly packed, it was difficult to directly count the number of pyramidal neurons in the CA1 area from a $30\ \mu\text{m}$ -thick brain section, so we estimated neuronal density using a semi-quantitative method involving calculating the percentage of an area occupied by Nissl-stained neurons in an area of interest in the CA1 area. Although a stereological approach involving the counting of cells in a complete series of sections would provide additional data [25], calculating the cell number in representative brain sections yielded similar histological results to those reported in the literature [17].

2.7. Data analysis

Analysis of variance (ANOVA) repeated measures was used to analyze the rotarod test data. ANOVA followed by the least-significant difference (LSD) post hoc test was used to analyze the results of T-maze and elevated plus-maze tests and IL-2 data. ANOVA with LSD post hoc test and paired-samples *t*-test were used to analyze the object recognition test data. All results are expressed as the mean \pm SEM. The level of significance was defined as $P < 0.05$ (two-tailed).

3. Results

3.1. Rotarod test

ANOVA repeated measures revealed that motor function in the rotarod test had main effects of time ($F(7,371) = 62.43, P < 0.001$) and treatment ($F(4,53) = 10.17, P < 0.01$) and time-by-treatment interactions ($F(28,371) = 4.78, P < 0.001$). Further analysis of the data for different time points by ANOVA showed that, compared to the sham-operated group, the percentage of the maximum time

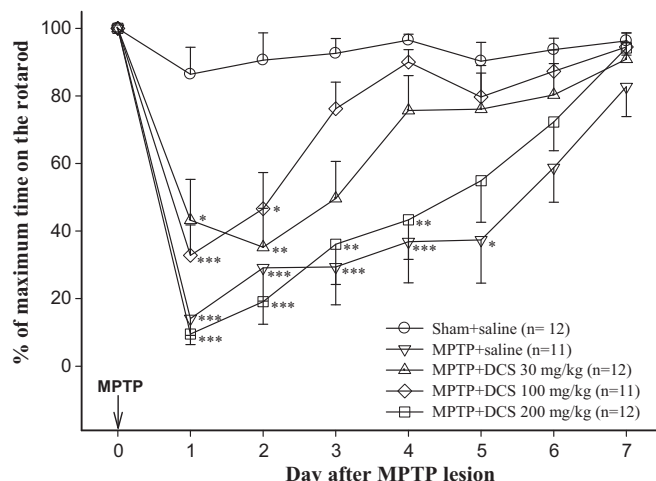


Fig. 1. Effects of D-cycloserine (DCS) on motor function in MPTP-lesioned rats in the rotarod test. MPTP ($1\ \mu\text{mol}$) was bilaterally infused into the substantia nigra pars compacta, then DCS (30, 100, and 200 mg/kg/day, i.p.) or saline (1 ml/kg/day, i.p.) was administered from day 1 after MPTP lesioning. The data are expressed as the mean \pm SEM for the indicated number of rats. * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$, compared to the sham-operated group on the same day.

on the rotarod in the MPTP-lesioned group was significantly lower on days 1–4 (all P values < 0.001) and on day 5 ($P < 0.05$) after MPTP lesioning, but not on days 6 and 7. Daily treatment with DCS restored the MPTP-induced motor deficits starting on day 3 at the dosages of 30 and 100 mg/kg/day and at day 5 at the dosage of 200 mg/kg/day (Fig. 1).

3.2. T-maze

ANOVA followed by LSD post hoc test revealed that MPTP lesioning decreased the percentage of correct responses in the T-maze test ($F(4,57) = 6.59, P < 0.001$), compared to the sham-operated group. All dosages of DCS used (30, 100, and 200 mg/kg/day) were able to reverse this deficit (Fig. 2). Further analysis, by using one-sample *t*-test, showed that the percentage of correct response in sham-operated rats and rats receiving MPTP lesion accompanied with DCS treatment was significantly higher than the chance level (50%) ($df = 10$ or 11 , all t values ≥ 7.31 , all P values ≤ 0.001). How-

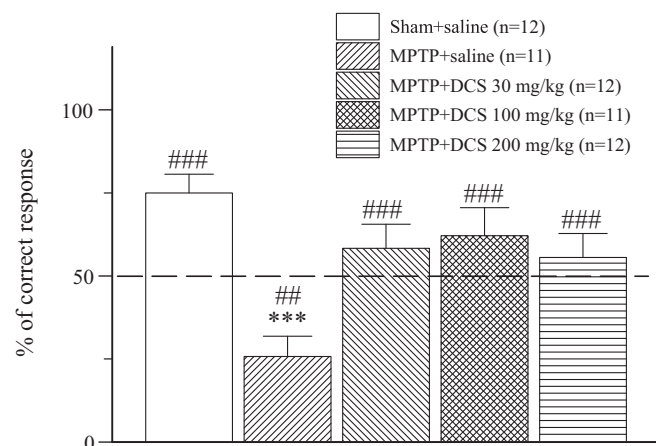


Fig. 2. Effects of D-cycloserine (DCS) on the behavior of MPTP-lesioned rats in the T-maze test. Animals were treated as in Fig. 1, then the T-maze test was performed on day 10 after MPTP lesioning. The data are expressed as the mean \pm SEM. *** $P < 0.001$, compared to the sham-operated control. ## $P < 0.01$, ### $P < 0.001$, compared to the chance level (50%).

Table 1
Effects of D-cycloserine on the behavior of MPTP-lesioned rats in the elevated plus-maze test.

| | Sham | MPTP | | | |
|-----------------------------|---------------|---------------|---------------------|----------------------|----------------------|
| | Saline (n=21) | Saline (n=20) | DCS 30 mg/kg (n=21) | DCS 100 mg/kg (n=22) | DCS 200 mg/kg (n=21) |
| Open arm time (s) | 32.0 ± 5.2 | 10.5 ± 2.7** | 22.5 ± 6.5 | 33.1 ± 9.7 | 51.0 ± 8.3 |
| Enclosed arm time (s) | 210.3 ± 13.9 | 208.1 ± 14.6 | 222.8 ± 10.4 | 221.0 ± 11.5 | 194.1 ± 12.0 |
| Enclosed arm activity (no.) | 11.0 ± 1.3 | 10.8 ± 1.5 | 10.5 ± 1.6 | 10.1 ± 1.6 | 9.6 ± 1.2 |
| Rearing (no.) | 9.3 ± 1.2 | 8.8 ± 1.1 | 8.1 ± 1.2 | 10.2 ± 0.9 | 9.3 ± 0.8 |

DCS: D-cycloserine. Data are expressed as the mean ± SEM. ** $P < 0.01$, compared to the sham-operated controls.

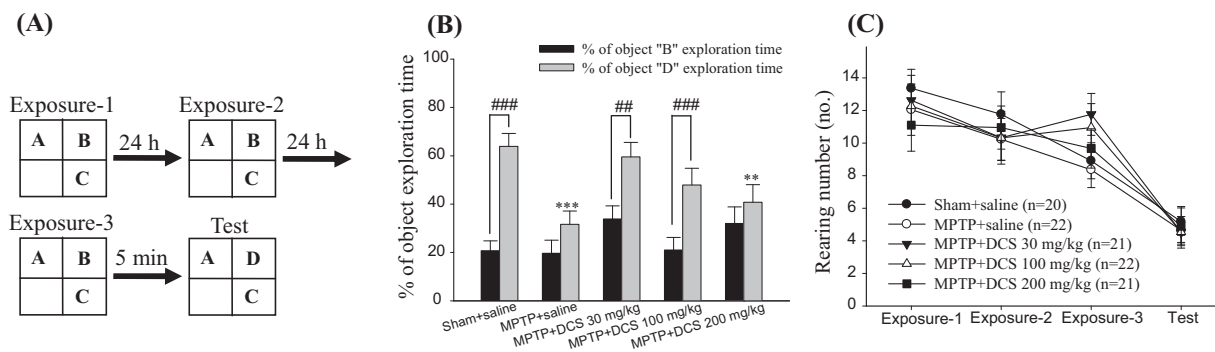


Fig. 3. Effects of D-cycloserine (DCS) on object recognition in MPTP-lesioned rats. Animals were treated as in Fig. 1, then the object recognition test was performed on days 11–13 after MPTP lesioning. (A) Schematic diagram of the arrangement of the objects in the test. Rats underwent 3 exposure sessions (5 min each) at 24 h intervals, then were tested for 5 min starting 5 min after the end of exposure session 3. In the test session, object “B” was replaced by a novel object “D”. (B) Percentage of time spent on exploring object “B” or “D”. (C) Rearing number in the exposure and test sessions. The data are expressed as the mean ± SEM. ** $P < 0.01$, *** $P < 0.001$, compared to the sham-operated group. ### $P < 0.01$, ### $P < 0.001$, compared to the percentage time spent on object “B” (paired t -test).

ever, MPTP-lesioned rats showed lowered percentage of correct response, compared to the chance level ($df = 10$, $t = 4.14$, $P = 0.002$).

Independent sample t -test showed no differences in the open arm time and enclosed arm time in the elevated plus-maze test ($df = 105$, t values ≤ 1.11 , both P values ≥ 0.27) and the percentage of exploration time spent on novel object in the object recognition test ($df = 104$, $t = 0.21$, $P = 0.835$) between the rats had and had not been tested in the rotarod and T-maze tests, indicating that the rotarod and T-maze tests did not affect the behavior in the followed elevated plus-maze test and object recognition test. Thus, the data of the flowed two tests from all of the rats, irrespective of receiving or not rotarod and T-maze tests, were analyzed.

3.3. Elevated plus-maze test

ANOVA with an LSD post hoc test indicated that MPTP lesioning significantly decreased open arm time ($F(4,106) = 4.66$, $P = 0.002$), compared to sham-operated controls. No deficit of open arm time

was observed in MPTP-lesioned rats treated with DCS at the dosage of 30, 100, and 200 mg/kg/day. No differences were observed in the enclosed arm time, enclosed arm activity, and rearing number between the groups (Table 1).

3.4. Object recognition

The method used in the test is shown in Fig. 3A. ANOVA revealed that there were no differences between the groups in total exploration time and the percentage of time exploring object “B” in the 3 exposure sessions (data not shown). ANOVA with LSD post hoc test showed that MPTP-lesioned rats receiving either saline or DCS 200 mg/kg spent a smaller percentage of time exploring object “D” ($F(4,105) = 4.73$, $P = 0.002$) than sham-operated controls. As shown in Fig. 3B, further analysis using the paired-samples t -test showed that the sham-operated and MPTP groups treated with DCS at the dosages of 30 and 100 mg/kg/day spent a higher percentage of time exploring object “D” than exploring object “B” ($df = 21$, t -values

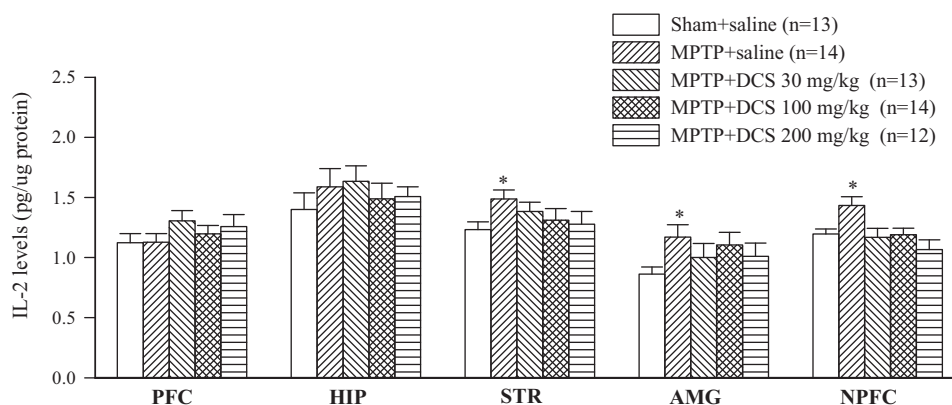


Fig. 4. Effects of daily treatment with D-cycloserine (DCS) on IL-2 levels in the brain of MPTP-lesioned rats. The brain tissue was taken 14 days after MPTP lesioning. The data are expressed as the mean ± SEM. * $P < 0.05$, compared to the sham-operated group.

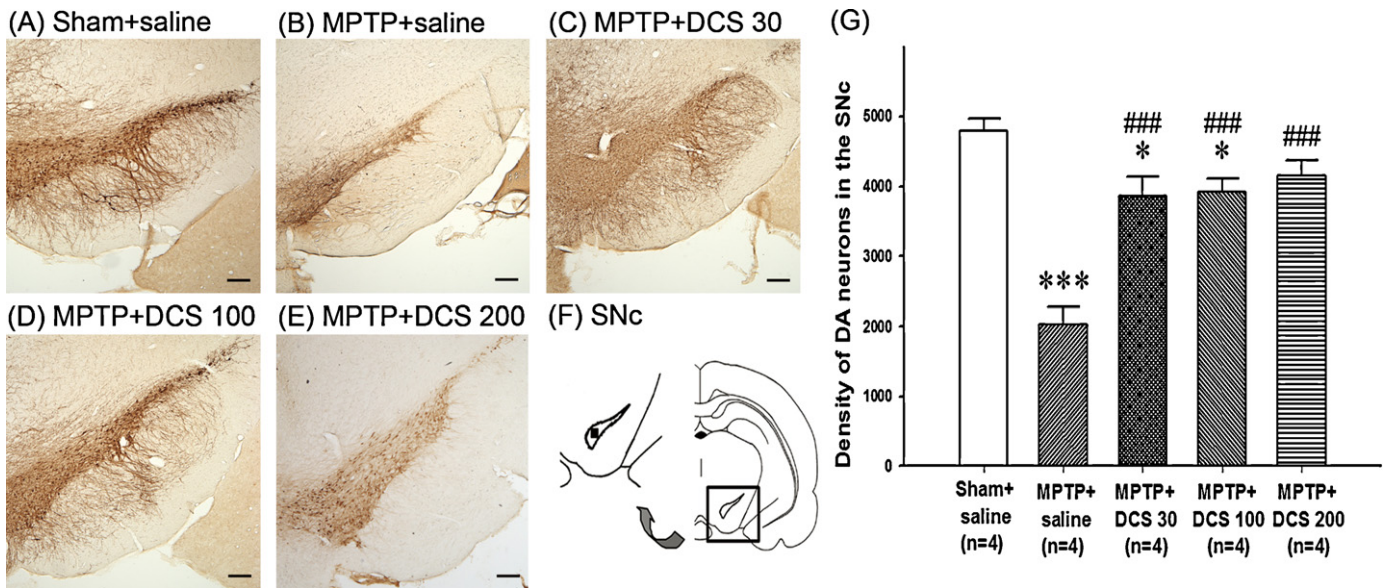


Fig. 5. Effects of D-cycloserine (DCS) on MPTP-induced changes in dopaminergic neurons in the SNc at day 14 after lesioning. Dopaminergic neurons stained for tyrosine hydroxylase (TH) are shown in the representative coronal sections. DCS 30, 100, or 200 indicates the dosage of DCS used. Magnification, 50 \times ; bar, 200 μ m. The black square in the schematic drawings indicates the area used for measuring the density of dopaminergic neurons. * P <0.05, *** P <0.001, compared to the Sham + saline group. ### P <0.001, compared to the MPTP + saline group.

≥ 3.15 , all P values ≤ 0.005). However, no difference in the percentage of time exploring objects “B” and “D” was observed between the MPTP-lesioned group and the MPTP-lesioned group treated with 200 mg/kg/day of DCS. In terms of rearing number in the object recognition test, ANOVA with repeated measures revealed a significant time effect ($F(3,306) = 45.50$, $P < 0.001$), but no significant treatment effect or time-by-treatment interactions (Fig. 3C).

3.5. IL-2 measurement

As shown in Fig. 4, compared to the sham-operated group, the MPTP-lesioned group showed a significant increase in IL-2 levels in the striatum, amygdala, and non-prefrontal cortex ($F(4,65) \geq 3.74$, all P values < 0.05), but not in the prefrontal cortex and hippocampus. However, the above changes were not observed in the

MPTP-lesioned rats treated with DCS at dosage of 30, 100, and 200 mg/kg/day.

3.6. Histology

Representative photomicrographs of immunostained and Nissl-stained brain sections are shown in Figs. 5–9. TH immunoreactivity was observed in the cell bodies of DAergic neurons in the SNc and in DAergic processes in the striatum. ANOVA showed that rats in the MPTP-lesioned group exhibited a decreased density of DAergic neurons in the SNc ($F(4,19) = 21.41$, $P < 0.001$) (Fig. 5B and G) and a lower relative optical density of TH immunoreactivity in the striatum ($F(4,19) = 14.73$, $P < 0.001$) (Fig. 6B and G) compared to the sham-operated group. The MPTP-induced decrease in the density of DAergic neurons in the SNc compared to the sham-operated

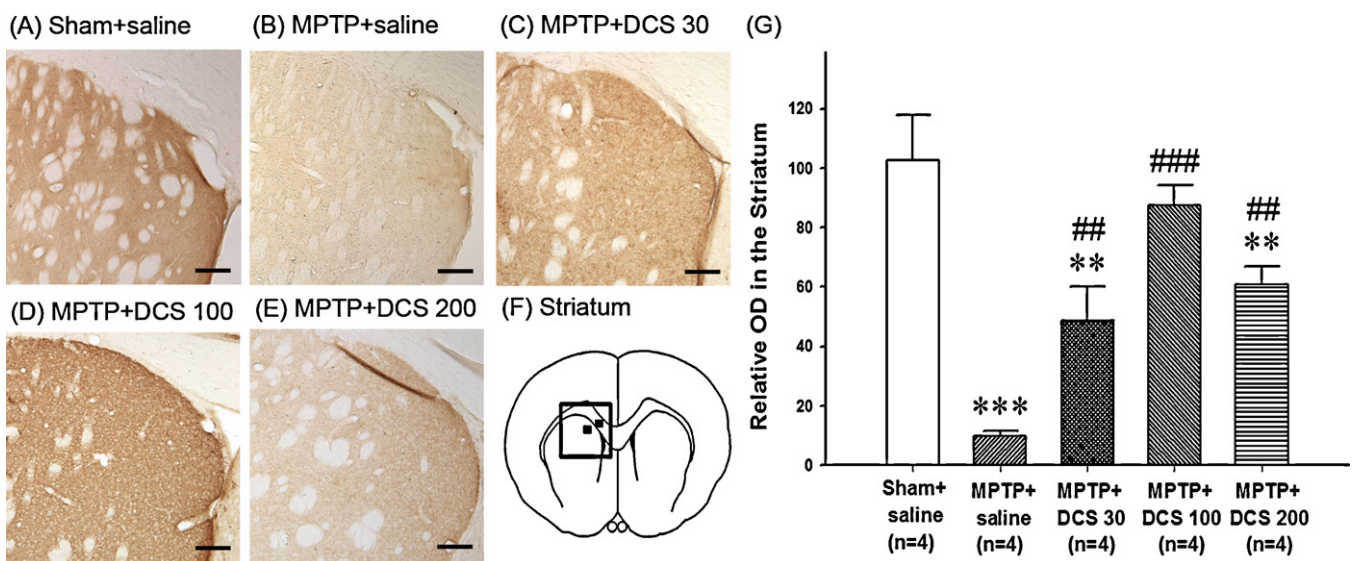


Fig. 6. Effects of D-cycloserine (DCS) on MPTP-induced changes of tyrosine hydroxylase (TH) immunoreactivity in the striatum at day 14 after lesioning. DCS 30, 100, or 200 indicates the dosage of DCS used. Magnification, 50 \times ; bar, 200 μ m. The black squares in the schematic drawing indicate the areas used for measuring optical density (OD). ** P <0.01, *** P <0.001, compared to the Sham + saline group. ## P <0.01, ### P <0.001, compared to the MPTP + saline group.

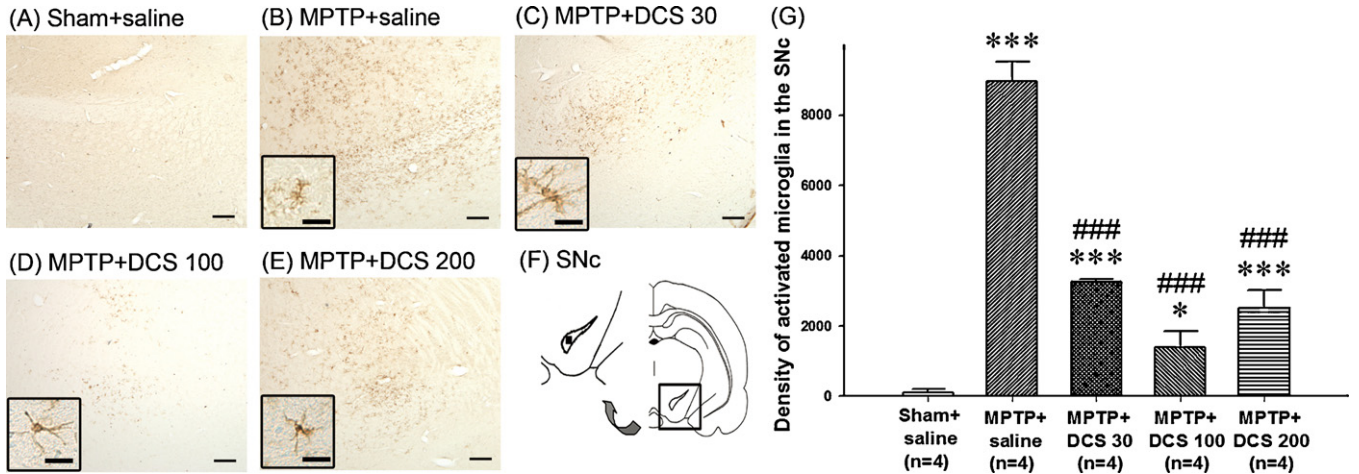


Fig. 7. Effects of D-cycloserine (DCS) on MPTP-induced activation of microglia in the SNc at day 14 after lesioning. DCS 30, 100, or 200 indicates the dosage of DCS used. Magnification, 50 \times ; bar, 200 μ m. A high magnification image (200 \times , bar, 20 μ m) of the activated microglia is shown in the insets. The black square in the schematic drawing indicates the area used for measuring the density of activated microglia in the SNc. * P <0.05, *** P <0.001, compared to the Sham + saline group. ### P <0.001, compared to the MPTP + saline group.

group was ameliorated by DCS treatment at 30 and 100 mg/kg/day (both P values <0.05) and totally prevented by DCS treatment at 200 mg/kg/day (Fig. 5C, D, E and G), while the MPTP-induced decrease in TH immunoreactivity in the striatum compared to the sham-operated group was ameliorated by DCS treatment at 30 and 200 mg/kg/day (both P values <0.01) and totally prevented at 100 mg/kg/day (Fig. 6C, D, E and G). Activated microglia, indicated by the accumulation of OX-6-positive cells, were detected after MPTP lesioning in the SNc (Fig. 7B) and striatum (Fig. 8B). In the SNc, ANOVA showed that the density of activated microglia in the MPTP-lesioned group was higher than that in the sham-operated control ($F(4,19)=75.73$, $P<0.001$) (Fig. 7B and G), and all three dosages of DCS ameliorated the MPTP-induced microglial activation compared to the MPTP-lesioned group (all P values <0.001) (Fig. 7C,

D, E and G). In the striatum, no activated microglia was found in MPTP-lesioned rats treated with DCS (Fig. 8C–E). In addition, neuronal density in the pyramidal cell layer in the hippocampal CA1 area was decreased in the MPTP-lesioned group compared to the sham-operated group ($F(4,19)=6.87$, $P=0.002$), and DCS treatment at 200 mg/kg/day ameliorated this difference and 30 and 100 mg/kg/day of DCS prevented it (Fig. 9).

4. Discussion

In the present study, MPTP lesioning caused disturbance of motor function, impairment of working memory in the T-maze test, anxiety-like behavior in the elevated plus-maze test, and deficits in object recognition, which were improved by daily treatment with

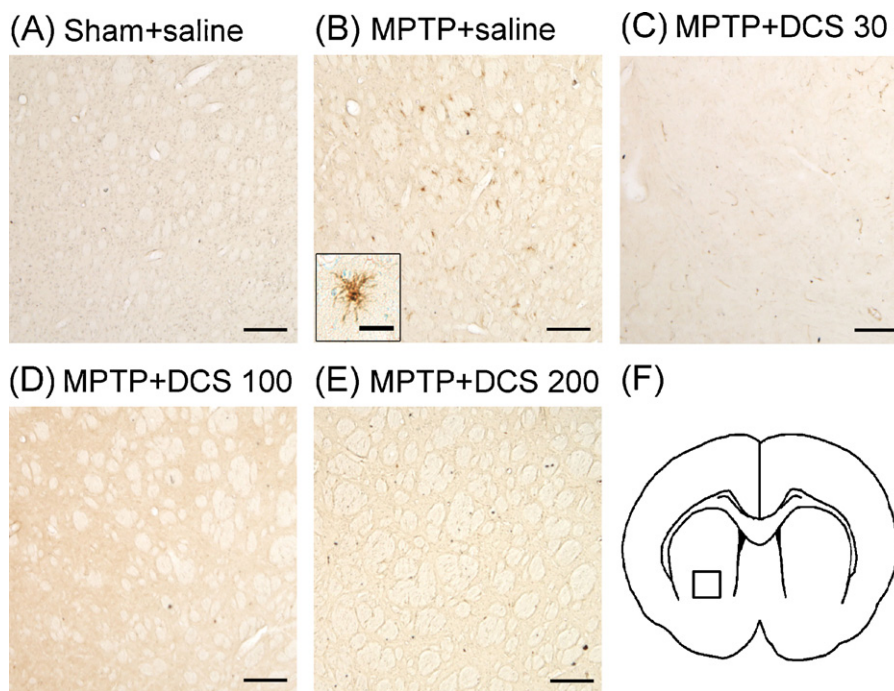


Fig. 8. Effects of D-cycloserine (DCS) on MPTP-induced activation of microglia in the striatum at day 14 after lesioning. DCS 30, 100, or 200 indicates the dosage of DCS used. Magnification, 50 \times ; bar, 200 μ m. A high magnification image (200 \times , bar, 20 μ m) of the activated microglia is shown in the inset. The square in the schematic drawings indicates the area used for measuring the density of activated microglia in the striatum.

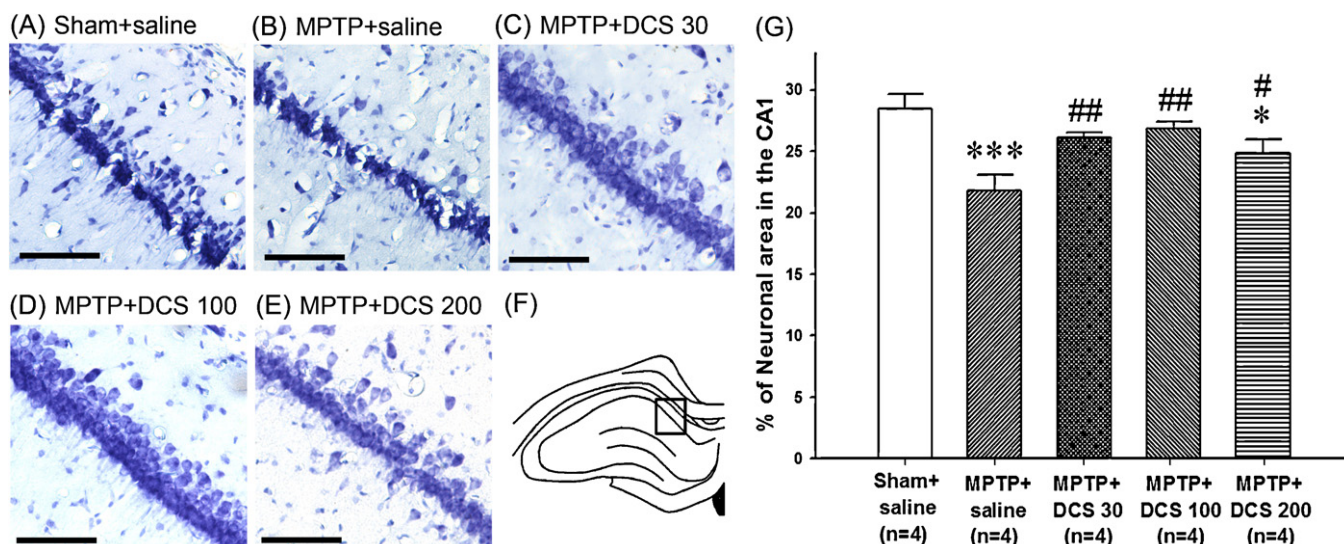


Fig. 9. Effects of D-cycloserine (DCS) on MPTP-induced cell loss in the hippocampal CA1 area at day 14 after lesioning. The images show Nissl-stained pyramidal neurons in the CA1 area of the hippocampus, as indicated in the square of the schematic drawing. DCS 30, 100, or 200 indicates the dosage of DCS used. Magnification, 200 \times ; bar, 100 μ m. * P <0.05, *** P =0.002, compared to the Sham + saline group. # P <0.05, ## P <0.01, compared to the MPTP + saline group.

DCS. MPTP lesioning also decreased density of DAergic neurons in the SNc and terminals in the striatum, caused massive activation of microglia in these two nuclei, decreased neuronal density in the CA1 area of the hippocampus, and increased IL-2 levels in the striatum, amygdala, and non-prefrontal cortex. The above neurological and biochemical changes were restored by DCS treatment. These results show that DCS overcomes the MPTP-induced deficits in motor, emotional, and cognitive behaviors, and reverses the neuroinflammation and neurodegeneration, suggesting a potential role for DCS in the treatment of behavioral and neuronal impairments in PDD.

In line with previous studies showing transient motor dysfunction during the first week after MPTP lesioning [13,25,86,92,98], the present study shows no differences in motor function in the rotarod test between the MPTP-lesioned and sham-operated groups at 6 days after surgery. Motor recovery was further supported by the lack of differences between the groups in enclosed arm activity and rearing number in the elevated plus-maze test and in rearing number in the object recognition test, suggesting that behavioral performance in the tests was not confounded by gross motor impairment or general sickness. Striatal re-innervation following lesions of SNc DAergic neurons may provide a possible compensatory mechanism of motor recovery in rats [90], although sustained decrease of TH-immunostaining in the striatum has been reported after MPTP lesion [9]. Even if no motor recovery has been seen in the progression of PD in humans, the ability of MPTP-lesioned rats to recover motor function makes it possible to study behavioral changes in these rats as a model for the motor presymptomatic phase of PD.

Because the effects of DCS vary depending on the activity of the glutamatergic system [99], we propose that a biphasic effect may occur in the DCS treatment, namely, during the acute phase after MPTP lesion, when the glutamatergic system is hyperactivated, DCS may act as an antagonist, while, in the later phase after MPTP lesion, the activity of the glutamatergic system may decrease and DCS may act as an agonist of NMDA receptor. MPTP lesioning results in an increase in corticostriatal glutamatergic innervation and regional reorganization of glutamatergic thalamostriatal terminals in the striatum of monkeys [79]. Ten days after MPTP lesioning, increased glutamate concentrations are seen in the striatum of mice [15]. Further, there was a decrease in the basal level of extracellular glutamate within the striatum in the sub-chronically MPTP-treated

animals compared to an increase in the acutely treated group [82]. Thus, we propose that, during the acute stage after MPTP lesioning, the glutamatergic system may be hyper-activated and thus cause excitotoxicity. However, after a period of time or in the chronic state, glutamatergic transmission may be hypo-activated, because the number of NMDA-sensitive [3 H]glutamate binding sites in MPTP-treated mice is decreased 2 weeks after MPTP lesioning [101]. Based on the above data, a biphasic evolution of glutamatergic activity may appear after MPTP lesioning in the substantia nigra. Thus, there may be a turning point, where the glutamatergic tonus changes from hyper- to hypo-activity, and this point may provide a basis for determining a temporal adequacy of treatment for these two phases. However, to our knowledge, no paper has reported this turning point. Hyper-glutamatergic activity induced by MPTP lesion has been implicated in excitotoxic neuronal death in the PD model [15,65,77,79], which may be involved in cell loss in the DAergic system and microglial activation in the hippocampus of PD brains [40]. Accordingly, treatment with NMDA antagonists before and immediately after MPTP lesioning can protect DAergic neurons from MPTP-induced degeneration [83,94] and improve motor function and suppress the progression of PD [8]. Similar to the above findings, our present study provides the first data showing that chronic treatment of DCS suppressed microglial activation and protected neurodegeneration in the hippocampus and nigrostriatal system from MPTP lesioning. Since DCS acts as an antagonist at the NMDA receptors when the glutamate levels are high [99], this drug may be able to prevent overstimulation of the glutamatergic system by excessively released glutamate after MPTP lesioning. This function, especially at the acute phase after MPTP lesioning, may thus have achieved neuroprotection from excitotoxicity [65,77], and caused a sustained benefit for the entire observation period. It may also be possible that, at the chronic state, DCS exerts cognition enhancing effect through the mechanisms described below.

The NMDA receptors play an important role in cognitive function, for example, spatial learning [56] and visual recognition memory [57]. DCS has promnesic effects in rats in learning tasks [38,52] and has been reported to facilitate correct responses in spatial recognition in the radial-arm maze in MK-801-treated rats [43]. Furthermore, DCS improves memory in aging rats [6,7], overcomes brain damage-induced impairments in LTP and object recognition in rats [103], and enhances episodic-like memory in MPTP-lesioned

rats [96]. MPTP-induced glutamatergic dysfunction has been suggested to be involved in cognitive dysfunction after MPTP lesioning [18,25,26,60,84]. DCS may ameliorate neuronal and behavioral deficits by regulating the activity of glutamatergic NMDA receptors. As a partial agonist, DCS binds to the glycine binding site of the NMDA receptors [37] and modulates the binding of glutamate to these receptors [87,99]. DCS activates NMDA receptors, but is less effective than the endogenous ligand, glycine [87,99], therefore, at low dose, DCS increases glutamatergic transmission when glutamate is at a physiological or low concentration [87], while, at high dose, it decreases the activation of NMDA receptors by glutamate [50]. DCS at dosages of 0.5–30 mg/kg has been reported to be effective in behavioral studies in rats [33,49]. In the present study, 30 and 100, but not 200, mg/kg/day of DCS reversed the MPTP-induced impairment of object recognition. Interestingly, the effects of DCS on motor function in MPTP-lesioned rats also showed a reversed U-shaped dose response curve, which may be due to the characteristics of the partial agonist, mimicking the activity of the endogenous ligand at low dose, but antagonizing it at a high dose [50]. However, all the dosages used in this study (30, 100, and 200 mg/kg/day) were effective on working memory in the T-maze test and anxiety-like behavior in the elevated plus-maze test and also restored IL-2 levels and microglial activation in the brain. Thus, there may be different effective-dose windows for different measurements. This hypothesis is supported by the findings that the behavioral and biochemical effects of DCS are related to neurophysiological activity in animals [33,100] and that the DCS-induced augmentation of effects of psychotherapy is seen in patients with anxiety disorders [35,80], but not in healthy controls [30].

The T-maze test is a kind of delayed alteration test, in which the rats need to learn a rule to make a correct choice, i.e. to choose the arm that was previously closed in forced run. Since the side of blockade of arm in forced runs was changed at random, the choice and performance in the T-maze test is regarded as working memory because of its trial-dependency [5]. The object recognition test is a kind of delayed matching to sample task [23] and is similar to the visual recognition task used in subhuman primates to assess memory dysfunction [3]. Primates and rodents show a tendency to explore new objects when a new object and a familiar object are presented together and they spend longer exploring a new object than a familiar object. Thus, the difference in the time spent exploring new and familiar objects is a measure of object recognition and/or discrimination in rats [23]. In the present study, sham-operated rats performed around 75% better than the chance level of 50% in correct responses in the T-maze test and showed successful discrimination in the object recognition test. However, MPTP lesioning significantly suppressed behavior in the T-maze and object recognition tests, indicating impairment of working memory and object recognition. Similarly, previous studies have shown that MPTP causes cognitive deficits in animals, including learning impairment in the two-way active avoidance test [17,26] and disturbance in spatial working memory and cue-based navigation, but sparing long-term spatial memory, in the Morris water maze test in rats [18,25,60], as well as a decrease in recognition response in the variable delayed-response task in monkeys [84]. It has been reported that a high percentage of patients with PDD has emotional symptoms [10,53] and that cognitively deteriorated PD patients perform more poorly in discriminating objects than healthy controls and cognitively preserved PD patients [47]. Thus, in addition to anxiety-like behavior in the elevated plus-maze test, the MPTP lesion-induced behavioral deficits in T-maze and object recognition tests may be similar to the anxiety, amnesia, and agnosia seen in PDD patients. These data suggest that MPTP-lesioned rats may not only be a model for amnesia [73], but also for PDD [92,98]. Since DCS treatment significantly reversed the MPTP-induced behavioral deficits and since DCS has also been implicated

as a “cognitive enhancer” in humans [36], this drug may have clinical potential in the treatment of PDD.

The hippocampus is essential for spatial navigation [106], recognition memory [11], working memory [39,85], and short-term memory associating objects and their locations [76]. Hippocampal dysfunction has been implicated in the visuospatial deficits observed in PD patients [27]. In agreement with previous reports [92,96,98], we showed that MPTP lesioning caused neuroinflammation in the brain and loss of pyramidal neurons in the hippocampal CA1 area. The decrease in neuronal density in the CA1 area may be involved in the MPTP-induced deficits in object recognition, as the improvement in this behavior after DCS treatment at the dosages of 30 and 100 mg/kg was accompanied by a protection of CA1 pyramidal cells from death. Similarly, enhancement of cognitive function by chronic DCS treatment at the dose of 30 mg/kg/day has also been observed in traumatic brain-injured-rats in the Morris water maze test [93], which requires intact hippocampal function [21]. In addition, acute treatment of DCS at the dose of 12 mg/kg increased the working memory in hippocampal-lesioned rats [85]. A previous study has reported that the involvement of hippocampal CA1 area in object recognition is delay-dependent because temporary inhibition of neuronal activity in this area impaired novel object preference in mice, after a 24 h but not a 5 min retention interval, indicating that the hippocampus is involved in encoding and/or retention of long-term object memory [31]. Since, in the current study, the intervals between object exposure sessions were 24 h, and the delay before novel object testing was 5 min, the treatment of DCS may have improved both encoding and retention of object memory. Moreover, the DCS-induced attenuation of neuroinflammation and restoration of the DAergic system may contribute to the preservation of motor and emotional behaviors and working memory in MPTP-lesioned rats, as the restoration of these behaviors by DCS treatment at all dosages used was associated with suppression of both microglial activation and the increase in IL-2 levels in the brain and with restoration of the density of DAergic neurons and terminals in the nigrostriatal system.

Clinical studies on PD patients have shown that IL-2 levels are increased in the SNc [63], caudate nucleus [61], and CSF [62]. MPTP is known to increase levels of cytokines [64,66], including IL-2 [98], in several brain regions in rodents, and this effect has been proposed to be involved in neuronal cell death in the DAergic system [64]. The present data showed that MPTP lesioning caused not only massive microglial activation, but also a widespread increase in IL-2 levels in the striatum, amygdala, and non-prefrontal cortex. DAergic degeneration in PD also causes microglial activation [68], which may be involved in the pathophysiological processes of PD by releasing inflammatory cytokines and leading to cell death [67]. Microglial activation is observed from day 1 to day 14 after MPTP-induced degeneration of DAergic neurons in the substantia nigra in mice [45], during which period the concentration of inflammatory cytokines, for example, IL-10 and IL-12, in the CSF is increased [104]. Degenerated neurons lead to further activation of microglia and aggravate the pathological pathway [97]. In addition, IL-2 is involved in cognitive function and neuronal development in the hippocampus [75] and plays a role in psychiatric disorders [55,58]. IL-2 immunotherapy elicits cognitive impairment [14,19], and repeated injection of IL-2 induces abnormalities in novelty-induced locomotion, learning, and spatial memory in the Morris water-maze test and altered exploratory activity in rodents [48,105]. Further, another study [74] and our previous studies [34,51] have shown that IL-2 levels are related to emotional behavior and that levels of striatal IL-2 mRNA are correlated with anxiety-like behavior in the elevated plus-maze test [70]. Striatal IL-2 microinjections can modulate anxiety-like behavior in the elevated plus-maze test [71] and induce avoidance behavior in the open field test [42]. Moreover, since the MPTP-induced cytokine

increase in the CSF of mice is observed during the first 2 weeks after lesioning [104] and since our animals were sacrificed one day after the last behavioral test on day 13 after MPTP lesioning, the IL-2 changes in this study were seen during the chronic state. All dosages of DCS used in this study corrected the deficits of anxiety-like behavior and working memory in MPTP-lesioned rats and this was associated with restoration of IL-2 levels in the brain, suggesting that the increase in IL-2 levels may be involved in the MPTP-induced behavioral deficits, notwithstanding the fact that other factors are probably also involved. In addition, the anti-inflammatory effects of DCS, such as decreasing microglial activation, may also underlie the behavioral effects, in agreement with a previous report suggesting that inhibition of neuroinflammation is an important strategy for preventing cognitive decline in PD [89].

Antagonists of NMDA receptor, for example, amantadine [29] and ketamine [24] have been demonstrated to protect DAergic neurons in the SNc in an MPTP-induced PD animal model. Although amantadine has been used in the clinic for the treatment of PD [28] and Alzheimer's disease [41], its clinical application is still controversial [78] because NMDA receptors are involved in a number of important neurobehavioral functions. This study showed that partial agonist of the NMDA receptors, DCS, may be a promising candidate for PD treatment. Further, the biphasic changes in glutamatergic activity after MPTP lesioning can also explain why chronic treatment of DCS at low dose (30 and 100 mg/kg), may through its agonistic activity on NMDA receptors, caused better effects than higher dose (200 mg/kg) on motor function, object recognition, and density of CA1 neurons in the current study. These data suggest that, at higher dose, DCS may have antagonistic effects on NMDA receptors, leading to a reduction in, or even lack of, efficacy. A previous study provides supports for this view, as a single administration of DCS at low dose (0.32 and 1.0 mg/kg) significantly improved spatial short-term memory during the chronic stage in MPTP-lesioned monkeys, but a higher dose (8 mg/kg) inhibited visual discrimination [84]. Thus, our study provides support for the low-dose strategy when using DCS in the clinic [35,80].

5. Conclusions

The present study shows that DCS treatment improves MPTP-induced deficits in motor, emotional, and cognitive behaviors and restores neuroinflammatory and neurodegenerative changes, suggesting its potential application in the prevention of the neuronal changes and dementia associated with PD. Furthermore, DCS has a good safety profile and is already in use in humans for several indications [20,32,46] and this could significantly facilitate its clinical application in PD patients.

Conflict of interest

The authors declare that there is no actual or potential conflict of interest in relation to this article.

Acknowledgements

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References

- [1] Aarsland D, Andersen K, Larsen JP, Lolk A, Nielsen H, Kragh-Sorensen P. Risk of dementia in Parkinson's disease: a community-based, prospective study. *Neurology* 2001;56:730–6.

- [2] Abramsky O, Litvin Y. Autoimmune response to dopamine-receptor as a possible mechanism in the pathogenesis of Parkinson's disease and schizophrenia. *Perspect Biol Med* 1978;22:104–14.
- [3] Aggleton JP, Mishkin M. Visual recognition impairment following medial thalamic lesions in monkeys. *Neuropsychologia* 1983;21:189–97.
- [4] Ando S, Kobayashi S, Waki H, Kon K, Fukui F, Tadenuma T, et al. Animal model of dementia induced by entorhinal synaptic damage and partial restoration of cognitive deficits by BDNF and carnitine. *J Neurosci Res* 2002;70:519–27.
- [5] Ando S, Ohashi Y. Longitudinal study on age-related changes of working and reference memory in the rat. *Neurosci Lett* 1991;128:17–20.
- [6] Baxter MG, Lanthorn TH, Frick KM, Golski S, Wan RQ, Olton DS. D-Cycloserine, a novel cognitive enhancer, improves spatial memory in aged rats. *Neurobiol Aging* 1994;15:207–13.
- [7] Billard JM, Rouaud E. Deficit of NMDA receptor activation in CA1 hippocampal area of aged rats is rescued by D-cycloserine. *Eur J Neurosci* 2007;25:2260–8.
- [8] Bonuccelli U, Del Dotto P. New pharmacologic horizons in the treatment of Parkinson disease. *Neurology* 2006;67:S30–8.
- [9] Bortolanza M, Wietzikoski EC, Boschen SL, Dombrowski PA, Latimer M, McLaren DA, et al. Functional disconnection of the substantia nigra pars compacta from the pedunculopontine nucleus impairs learning of a conditioned avoidance task. *Neurobiol Learn Mem* 2010;94:229–39.
- [10] Bosboom JL, Stoffers D, Wolters E. Cognitive dysfunction and dementia in Parkinson's disease. *J Neural Transm* 2004;111:1303–15.
- [11] Broadbent NJ, Squire LR, Clark RE. Spatial memory, recognition memory, and the hippocampus. *Proc Natl Acad Sci USA* 2004;101:14515–20.
- [12] Brown RG, Marsden CD. How common is dementia in Parkinson's disease? *Lancet* 1984;2:1262–5.
- [13] Capitelli C, Sereniki A, Lima MM, Reksidler AB, Tufik S, Vital MA. Melatonin attenuates tyrosine hydroxylase loss and hypolocomotion in MPTP-lesioned rats. *Eur J Pharmacol* 2008;594:101–8.
- [14] Caraceni A, Martini C, Belli F, Mascheroni L, Rivoltini L, Arienti F, et al. Neuropsychological and neurophysiological assessment of the central effects of interleukin-2 administration. *Eur J Cancer* 1993;29A:1266–9.
- [15] Chassain C, Bielicki G, Durand E, Lolignier S, Essafi F, Traore A, et al. Metabolic changes detected by proton magnetic resonance spectroscopy in vivo and in vitro in a murin model of Parkinson's disease, the MPTP-intoxicated mouse. *J Neurochem* 2008;105:874–82.
- [16] Crucian GP, Okun MS. Visual-spatial ability in Parkinson's disease. *Front Biosci* 2003;8:s992–7.
- [17] Da Cunha C, Gevaerd MS, Vital MA, Miyoshi E, Andreatini R, Silveira R, et al. Memory disruption in rats with nigral lesions induced by MPTP: a model for early Parkinson's disease amnesia. *Behav Brain Res* 2001;124:9–18.
- [18] Da Cunha C, Wietzikoski S, Wietzikoski EC, Miyoshi E, Ferro MM, Anselmo-Franci JA, et al. Evidence for the substantia nigra pars compacta as an essential component of a memory system independent of the hippocampal memory system. *Neurobiol Learn Mem* 2003;79:236–42.
- [19] Denicoff KD, Rubinow DR, Papa MZ, Simpson C, Seipp CA, Lotze MT, et al. The neuropsychiatric effects of treatment with interleukin-2 and lymphokine-activated killer cells. *Ann Intern Med* 1987;107:293–300.
- [20] Duncan EJ, Szilagyi S, Schwartz MP, Bugarski-Kirola D, Kunzova A, Negi S, et al. Effects of D-cycloserine on negative symptoms in schizophrenia. *Schizophr Res* 2004;71:239–48.
- [21] Duva CA, Floresco SB, Wunderlich GR, Lao TL, Pineda JP, Phillips AG. Disruption of spatial but not object-recognition memory by neurotoxic lesions of the dorsal hippocampus in rats. *Behav Neurosci* 1997;111:1184–96.
- [22] Emre M. Dementia associated with Parkinson's disease. *Lancet Neurol* 2003;2:229–37.
- [23] Ennaceur A, Delacour J. A new one-trial test for neurobiological studies of memory in rats. 1: behavioral data. *Behav Brain Res* 1988;31:47–59.
- [24] Ferro MM, Angelucci ME, Anselmo-Franci JA, Canteras NS, Da Cunha C. Neuroprotective effect of ketamine/xylazine on two rat models of Parkinson's disease. *Braz J Med Biol Res* 2007;40:89–96.
- [25] Ferro MM, Bellissimo ML, Anselmo-Franci JA, Angelucci ME, Canteras NS, Da Cunha C. Comparison of bilaterally 6-OHDA- and MPTP-lesioned rats as models of the early phase of Parkinson's disease: histological, neurochemical, motor and memory alterations. *J Neurosci Meth* 2005;148:78–87.
- [26] Gevaerd MS, Takahashi RN, Silveira R, Da Cunha C. Caffeine reverses the memory disruption induced by intra-nigral MPTP-injection in rats. *Brain Res Bull* 2001;55:101–6.
- [27] Girotti F, Soliveri P, Carella F, Piccolo I, Caffarra P, Musicco M, et al. Dementia and cognitive impairment in Parkinson's disease. *J Neurol Neurosurg Psychiatry* 1988;51:1498–502.
- [28] Goetz CG. New lessons from old drugs: amantadine and Parkinson's disease. *Neurology* 1998;50:1211–2.
- [29] Greenamyre JT, O'Brien CF. N-Methyl-D-aspartate antagonists in the treatment of Parkinson's disease. *Arch Neurol* 1991;48:977–81.
- [30] Guastella AJ, Dadds MR, Lovibond PF, Mitchell P, Richardson R. A randomized controlled trial of the effect of D-cycloserine on exposure therapy for spider fear. *J Psychiatr Res* 2007;41:466–71.
- [31] Hammond RS, Tull LE, Stackman RW. On the delay-dependent involvement of the hippocampus in object recognition memory. *Neurobiol Learn Mem* 2004;82:26–34.
- [32] Heresco-Levy U, Kremer I, Javitt DC, Goichman R, Reshef A, Blarum M, et al. Pilot-controlled trial of D-cycloserine for the treatment of post-traumatic stress disorder. *Int J Neuropsychopharmacol* 2002;5:301–7.

- [33] Ho YJ, Hsu LS, Wang CF, Hsu WY, Lai TJ, Hsu CC, et al. Behavioral effects of D-cycloserine in rats: the role of anxiety level. *Brain Res* 2005;1043:179–85.
- [34] Ho YJ, Wang CF, Hsu WY, Tseng T, Hsu CC, Kao MD, et al. Psychoimmunological effects of dioscorea in ovariectomized rats: role of anxiety level. *Ann Gen Psychiatry* 2007;6:21–8.
- [35] Hofmann SG, Meuret AE, Smits JA, Simon NM, Pollack MH, Eisenmenger K, et al. Augmentation of exposure therapy with D-cycloserine for social anxiety disorder. *Arch Gen Psychiatry* 2006;63:298–304.
- [36] Hofmann SG, Pollack MH, Otto MW. Augmentation treatment of psychotherapy for anxiety disorders with D-cycloserine. *CNS Drug Rev* 2006;12:208–17.
- [37] Hood WF, Compton RP, Monahan JB. D-Cycloserine: a ligand for the N-methyl-D-aspartate coupled glycine receptor has partial agonist characteristics. *Neurosci Lett* 1989;98:91–5.
- [38] Hughes RN. Responsiveness to brightness change in male and female rats following treatment with the partial agonist of the N-methyl-D-aspartate receptor, D-cycloserine. *Behav Brain Res* 2004;152:199–207.
- [39] Hunsaker MR, Thorup JA, Welch T, Kesner RP. The role of CA3 and CA1 in the acquisition of an object-trace-place paired-associate task. *Behav Neurosci* 2006;120:1252–6.
- [40] Imamura K, Hishikawa N, Sawada M, Nagatsu T, Yoshida M, Hashizume Y. Distribution of major histocompatibility complex class II-positive microglia and cytokine profile of Parkinson's disease brains. *Acta Neuropathol* 2003;106:518–26.
- [41] Inzelberg R, Bonuccelli U, Schechtman E, Miniowich A, Strugatsky R, Ceravolo R, et al. Association between amantadine and the onset of dementia in Parkinson's disease. *Mov Disord* 2006;21:1375–9.
- [42] Karrenbauer BD, Ho YJ, Ludwig W, Löhning J, Spanagel R, Schwarting RK, et al. Time-dependent effects of striatal interleukin-2 on open field behaviour in rats. *J Neuroimmunol* 2009;208:10–8.
- [43] Kawabe K, Yoshihara T, Ichitani Y, Iwasaki T. Intrahippocampal D-cycloserine improves MK-801-induced memory deficits: radial-arm maze performance in rats. *Brain Res* 1998;814:226–30.
- [44] Kim YS, Joh TH. Microglia, major player in the brain inflammation: their roles in the pathogenesis of Parkinson's disease. *Exp Mol Med* 2006;38:333–47.
- [45] Kohutnicka M, Lewandowska E, Kurkowska-Jastrzebska I, Czlonkowska A, Czlonkowska A. Microglial and astrocytic involvement in a murine model of Parkinson's disease induced by 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP). *Immunopharmacology* 1998;39:167–80.
- [46] Laake K, Oeksengaard AR. D-Cycloserine for Alzheimer's disease. *Coch Data Sys Rev* 2002;CD003153.
- [47] Laatu S, Revonsuo A, Pihko L, Portin R, Rinne JO. Visual object recognition deficits in early Parkinson's disease. *Parkinsonism Relat Disord* 2004;10:227–33.
- [48] Lacosta S, Merali Z, Anisman H. Influence of acute and repeated interleukin-2 administration on spatial learning, locomotor activity, exploratory behaviors, and anxiety. *Behav Neurosci* 1999;113:1030–41.
- [49] Land C, Riccio DC. D-cycloserine: effects on long-term retention of a conditioned response and on memory for contextual attributes. *Neurobiol Learn Mem* 1999;72:158–68.
- [50] Lanthorn TH. D-Cycloserine: agonist turned antagonist. *Amino Acids* 1994;6:247–60.
- [51] Lee YT, Wang WF, Cheng CW, Wu SL, Pawlak CR, Ho YJ. Effects of escapable and inescapable stressors on behavior and interleukin-2 in the brain. *Neuroreport* 2008;19:1243–7.
- [52] Lelong V, Dauphin F, Boulouard M. RS 67333 and D-cycloserine accelerate learning acquisition in the rat. *Neuropharmacology* 2001;41:517–22.
- [53] Levin BE, Llabre MM, Reisman S, Weiner WJ, Sanchez-Ramos J, Singer C, et al. Visuospatial impairment in Parkinson's disease. *Neurology* 1991;41:365–9.
- [54] Li JS, Chao YS. Electrolytic lesions of dorsal CA3 impair episodic-like memory in rats. *Neurobiol Learn Mem* 2008;89:192–8.
- [55] Licinio J, Seibyl JP, Altemus M, Charney DS, Krystal JH. Elevated CSF levels of interleukin-2 in neuroleptic-free schizophrenic patients. *Am J Psychiatry* 1993;150:1408–10.
- [56] Mandillo S, Rinaldi A, Oliverio A, Mele A. Repeated administration of phenylcyclidine, amphetamine and MK-801 selectively impairs spatial learning in mice: a possible model of psychotomimetic drug-induced cognitive deficits. *Behav Pharmacol* 2003;14:533–44.
- [57] Matsuoka N, Aigner TG. D-Cycloserine, a partial agonist at the glycine site coupled to N-methyl-D-aspartate receptors, improves visual recognition memory in rhesus monkeys. *J Pharmacol Exp Ther* 1996;278:891–7.
- [58] McAllister CG, van Kammen DP, Rehn TJ, Miller AL, Gurklis J, Kelley ME, et al. Increases in CSF levels of interleukin-2 in schizophrenia: effects of recurrence of psychosis and medication status. *Am J Psychiatry* 1995;152:1291–7.
- [59] McGeer PL, McGeer EG. Inflammation and neurodegeneration in Parkinson's disease. *Parkinsonism Relat Disord* 2004;10(Suppl. 1):S3–7.
- [60] Miyoshi E, Wietzikoski S, Complessei M, Silveira R, Takahashi RN, Da Cunha C. Impaired learning in a spatial working memory version and in a cued version of the water maze in rats with MPTP-induced mesencephalic dopaminergic lesions. *Brain Res Bull* 2002;58:41–7.
- [61] Mogi M, Harada M, Kondo T, Riederer P, Nagatsu T. Interleukin-2 but not basic fibroblast growth factor is elevated in parkinsonian brain. *J Neural Transm* 1996;103:1077–81.
- [62] Mogi M, Harada M, Narabayashi H, Inagaki H, Minami M, Nagatsu T. Interleukin (IL)-1 beta, IL-2, IL-4, IL-6 and transforming growth factor-alpha levels are elevated in ventricular cerebrospinal fluid in juvenile parkinsonism and Parkinson's disease. *Neurosci Lett* 1996;211:13–6.
- [63] Mogi M, Harada M, Riederer P, Narabayashi H, Fujita K, Nagatsu T. Tumor necrosis factor-alpha (TNF-alpha) increases both in the brain and in the cerebrospinal fluid from parkinsonian patients. *Neurosci Lett* 1994;165:208–10.
- [64] Mogi M, Togari A, Ogawa M, Ikeguchi K, Shizuma N, Fan D, et al. Effects of repeated systemic administration of 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) to mice on interleukin-1beta and nerve growth factor in the striatum. *Neurosci Lett* 1998;250:25–8.
- [65] Mosley RL, Benner EJ, Kadiu I, Thomas M, Boska MD, Hasan K, et al. Neuroinflammation, oxidative stress and the pathogenesis of Parkinson's disease. *Clin Neurosci Res* 2006;6:261–81.
- [66] Nagatsu T, Mogi M, Ichinose H, Togari A. Cytokines in Parkinson's disease. *J Neural Transm Suppl* 2000:143–51.
- [67] Nakajima K, Kohsaka S. Microglia: neuroprotective and neurotrophic cells in the central nervous system. *Curr Drug Targets Cardiovasc Haematol Disord* 2004;4:65–84.
- [68] Ouchi Y, Yoshikawa E, Sekine Y, Futatsubashi M, Kanno T, Ogusu T, et al. Microglial activation and dopamine terminal loss in early Parkinson's disease. *Ann Neurol* 2005;57:168–75.
- [69] Owen AM, Sahakian BJ, Hodges JR, Summers BA, Polkey CE, Robbins TW. Dopamine-dependent frontostriatal planning deficits in early Parkinson's disease. *Neuropsychology* 1995;9:126–40.
- [70] Pawlak CR, Ho YJ, Schwarting RK, Bauhofer A. Relationship between striatal levels of interleukin-2 mRNA and plus-maze behavior in the rat. *Neurosci Lett* 2003;341:205–8.
- [71] Pawlak CR, Schwarting RK. Striatal microinjections of interleukin-2 and rat behaviour in the elevated plus-maze. *Behav Brain Res* 2006;168:339–44.
- [72] Paxinos G, Watson C. The rat brain in stereotaxic coordinates. London: Academic Press; 1986.
- [73] Perry JC, Hipolide DC, Tufik S, Martins RD, Da Cunha C, Andreatini R, et al. Intra-nigral MPTP lesion in rats: behavioral and autoradiography studies. *Exp Neurol* 2005;195:322–9.
- [74] Petito JM, McCarthy DB, Rinker CM, Huang Z, Getty T. Modulation of behavioral and neurochemical measures of forebrain dopamine function in mice by species-specific interleukin-2. *J Neuroimmunol* 1997;73:183–90.
- [75] Petito JM, McNamara RK, Gendreau PL, Huang Z, Jackson AJ. Impaired learning and memory and altered hippocampal neurodevelopment resulting from interleukin-2 gene deletion. *J Neurosci Res* 1999;56:441–6.
- [76] Piekema C, Kessels RP, Mars RB, Petersson KM, Fernandez G. The right hippocampus participates in short-term memory maintenance of object-location associations. *Neuroimage* 2006;33:374–82.
- [77] Plaitakis A, Shashidharan P. Glutamate transport and metabolism in dopaminergic neurons of substantia nigra: implications for the pathogenesis of Parkinson's disease. *J Neurol* 2000;247(Suppl. 2):II25–35.
- [78] Rajput A. Can amantadine therapy delay the onset of dementia in Parkinson's disease? *Nat Clin Pract Neurol* 2006;2:648–9.
- [79] Raju DV, Ahern TH, Shah DJ, Wright TM, Standaert DG, Hall RA, et al. Differential synaptic plasticity of the corticostriatal and thalamostriatal systems in an MPTP-treated monkey model of parkinsonism. *Eur J Neurosci* 2008;27:1647–58.
- [80] Ressler KJ, Rothbaum BO, Tannenbaum L, Anderson P, Graap K, Zimand E, et al. Cognitive enhancers as adjuncts to psychotherapy: use of D-cycloserine in phobic individuals to facilitate extinction of fear. *Arch Gen Psychiatry* 2004;61:1136–44.
- [81] Riekkinen M, Riekkinen PJ. Nicotine and D-cycloserine enhance acquisition of water maze spatial navigation in aged rats. *Neuroreport* 1997;8:699–703.
- [82] Robinson S, Freeman P, Moore C, Touchon JC, Krentz L, Meshul CK. Acute and subchronic MPTP administration differentially affects striatal glutamate synaptic function. *Exp Neurol* 2003;180:74–87.
- [83] schmiTabatabaei A, Perry TL, Hansen S, Krieger C. Partial protective effect of MK-801 on MPTP-induced reduction of striatal dopamine in mice. *Neurosci Lett* 1992;141:192–4.
- [84] Schneider JS, Tinker JP, Van Velson M, Giardinieri M. Effects of the partial glycine agonist D-cycloserine on cognitive functioning in chronic low dose MPTP-treated monkeys. *Brain Res* 2000;860:190–4.
- [85] Schuster GM, Schmidt WJ. D-Cycloserine reverses the working memory impairment of hippocampal-lesioned rats in a spatial learning task. *Eur J Pharmacol* 1992;224:97–8.
- [86] Sedelis M, Schwarting RK, Huston JP. Behavioral phenotyping of the MPTP mouse model of Parkinson's disease. *Behav Brain Res* 2001;125:109–25.
- [87] Sheinin A, Shavit S, Benveniste M. Subunit specificity and mechanism of action of NMDA partial agonist D-cycloserine. *Neuropharmacology* 2001;41:151–8.
- [88] Shi L, Adams MM, Long A, Carter CC, Bennett C, Sonntag WE, et al. Spatial learning and memory deficits after whole-brain irradiation are associated with changes in NMDA receptor subunits in the hippocampus. *Radiat Res* 2006;166:892–9.
- [89] Sriram K, Matheson JM, Benkovic SA, Miller DB, Luster MI, O'Callaghan JP. Deficiency of TNF receptors suppresses microglial activation and alters the susceptibility of brain regions to MPTP-induced neurotoxicity: role of TNF-alpha. *FASEB J* 2006;20:670–82.
- [90] Stanic D, Finkelstein DI, Bourke DW, Drago J, Horne MK. Time course of striatal re-innervation following lesions of dopaminergic SNpc neurons of the rat. *Eur J Neurosci* 2003;18:1175–88.

- [91] Stern Y, Tetrud JW, Martin WR, Kutner SJ, Langston JW. Cognitive change following MPTP exposure. *Neurology* 1990;40:261–4.
- [92] Sy HN, Wu SL, Wang WF, Chen CH, Huang YT, Liou YM, et al. MPTP-induced dopaminergic degeneration and deficits in object recognition in rats are accompanied by neuroinflammation in the hippocampus. *Pharmacol Biochem Behav* 2010;95:158–65.
- [93] Temple MD, Hamm RJ. Chronic, post-injury administration of D-cycloserine, an NMDA partial agonist, enhances cognitive performance following experimental brain injury. *Brain Res* 1996;741:246–51.
- [94] Turski L, Bressler K, Rettig KJ, Loschmann PA, Wachtel H. Protection of substantia nigra from MPP+ neurotoxicity by N-methyl-D-aspartate antagonists. *Nature* 1991;349:414–8.
- [95] Voss J, Sanchez C, Michelsen S, Ebert B. Rotarod studies in the rat of the GABAA receptor agonist gaboxadol: lack of ethanol potentiation and benzodiazepine cross-tolerance. *Eur J Pharmacol* 2003;482:215–22.
- [96] Wang AL, Liou YM, Pawlak CR, Ho YJ. Involvement of NMDA receptors in both MPTP-induced neuroinflammation and deficits in episodic-like memory in Wistar rats. *Behav Brain Res* 2010;208:38–46.
- [97] Wang T, Pei Z, Zhang W, Liu B, Langenbach R, Lee C, et al. MPP+-induced COX-2 activation and subsequent dopaminergic neurodegeneration. *FASEB J* 2005;19:1134–6.
- [98] Wang WF, Wu SL, Liou YM, Wang AL, Pawlak CR, Ho YJ. MPTP lesion causes neuroinflammation and deficits in object recognition in Wistar rats. *Behav Neurosci* 2009;123:1261–70.
- [99] Watson GB, Bolanowski MA, Baganoff MP, Deppeler CL, Lanthorn TH. D-Cycloserine acts as a partial agonist at the glycine modulatory site of the NMDA receptor expressed in *Xenopus* oocytes. *Brain Res* 1990;510:158–60.
- [100] Wu SL, Hsu LS, Tu WT, Wang WF, Huang YT, Pawlak CR, et al. Effects of D-cycloserine on the behavior and ERK activity in the amygdala: role of individual anxiety levels. *Behav Brain Res* 2008;187:246–53.
- [101] Wullner U, Brouillet E, Isacson O, Young AB, Penney JB. Glutamate receptor binding sites in MPTP-treated mice. *Exp Neurol* 1993;121:284–7.
- [102] Xavier LL, Viola GG, Ferraz AC, Da Cunha C, Deonizio JM, Netto CA, et al. A simple and fast densitometric method for the analysis of tyrosine hydroxylase immunoreactivity in the substantia nigra pars compacta and in the ventral tegmental area. *Brain Res Protoc* 2005;16:58–64.
- [103] Yaka R, Biegon A, Grigoriadis N, Simeonidou C, Grigoriadis S, Alexandrovich AG, et al. D-Cycloserine improves functional recovery and reinstates long-term potentiation (LTP) in a mouse model of closed head injury. *FASEB J* 2007;21:2033–41.
- [104] Yasuda Y, Shimoda T, Uno K, Tateishi N, Furuya S, Yagi K, et al. The effects of MPTP on the activation of microglia/astrocytes and cytokine/chemokine levels in different mice strains. *J Neuroimmunol* 2008;204:43–51.
- [105] Zalzman SS. Interleukin-2 potentiates novelty- and GBR 12909-induced exploratory activity. *Brain Res* 2001;899:1–9.
- [106] Zhang WN, Pothuizen HH, Feldon J, Rawlins JN. Dissociation of function within the hippocampus: effects of dorsal, ventral and complete excitotoxic hippocampal lesions on spatial navigation. *Neuroscience* 2004;127:289–300.