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Research report

Involvement of NMDA receptors in both MPTP-induced neuroinflammation and deficits in episodic-like memory in Wistar rats

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ABSTRACT

Hyper-activation of glutamatergic activity and deficits in episodic memory has been observed in Parkinson's disease (PD). This study was intended to clarify the effects of D-cycloserine (DCS), a partial agonist of N-methyl-D-aspartate (NMDA) receptors, on neuroinflammation and deficits in episodic-like memory in the 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP)-induced PD animal model. Male Wistar rats were stereotaxically administered with MPTP into the substantia nigra pars compacta. Starting 1 day after the lesion, animals were treated daily with DCS (0, 5, or 10 mg/kg/day; i.p.). Thirteen days after the MPTP lesion, the rats received the episodic-like memory test. Sham-operated rats exhibited episodic-like memory, recognizing objects' location and objects' temporal order. MPTP-lesioned rats exhibited deterioration in spatial memory and failed to recognize the temporal order of objects. Further, MPTP lesions resulted in dopaminergic degeneration and microglial activation in the brain, as well as cell loss in the hippocampal CA1 area. DCS treatment (10 mg/kg/day) reversed the above neurodegeneration, neuroinflammation, and behavioral deficits. Taken together, these results suggest that NMDA receptors may be involved in cognitive deficits in PD and that the application of DCS in the treatment for dementia in PD is warranted.

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1. Introduction

Defective episodic memory in story-recall tests has been demonstrated in patients with Parkinson's disease (PD), where the patients are not able to combine a recollection of past experience in terms of what happened, where it happened, and when it happened. 1-Methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP), a neurotoxin that selectively damages dopaminergic (DAergic) cells in the substantia nigra pars compacta (SNc), is widely used to induce animal models of PD in rodents and primates [14,43,54]. MPTP causes not only motor dysfunction and DAergic degeneration in the brain [5,12] but also neuroinflammation [32,33] and hyperactivity of the glutamatergic system [53]. In addition, cognitive impairment has been observed in MPTP-treated rats measured in relation to the two-way active avoidance task [14] and the Morris water maze test [8,12,43], as well as in monkeys tested in object-recognition tasks [54]. The above data suggest that neuroinflammation and hyper-glutamatergic activity may take part in

MPTP-induced deficits in learning, spatial, and temporal functions. However, connections between glutamatergic hyper-activation and cognitive deficits in PD are not yet understood.

MPTP-induced increases of glutamate release in the striatum [53], presumably via activation of N-methyl-D-aspartate (NMDA) receptors and causing a large influx of Ca^{2+} , induce neuronal excitotoxicity [48]. NMDA receptors in the hippocampus mediate not only learning and memory [55] but also object recognition [1,6,17,51]. Moreover, research has shown that NMDA receptor blockers suppress visuospatial ability [39], visual recognition [40], and spatial memory in the radial-arm maze task [31]. D-Cycloserine (DCS), a partial agonist at the glycine modulatory site on the NMDA receptor, is able to reverse memory function lost in aging rats [2,3] and impairments of recognition in MPTP-treated monkeys [54]. DCS can also offset brain damage-induced impairment of long-term potentiation in the hippocampus [65], and enhances episodic-like memory in mice [66]. And intrahippocampal injection of DCS has been reported to reverse MK-801-induced memory deficits in rats [31]. Episodic-like memory has been demonstrated in both rats and mice [9,30,37,66], where the animals have the ability to combine "what," "where," and "when" factors to form an integrated memory system. The aims of this study were, first, evaluating whether MPTP affects episodic-like memory in rats, and second,

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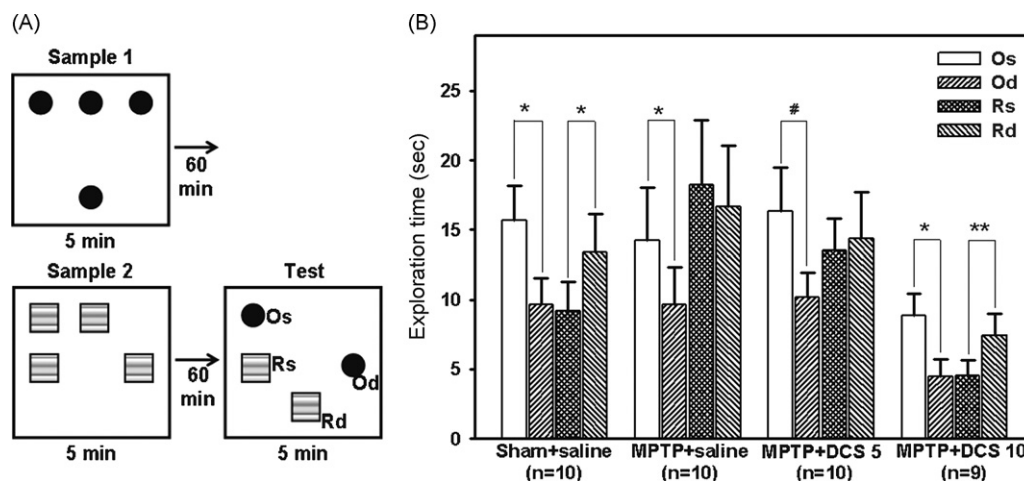


Fig. 1. Effects of D-cycloserine (DCS) on the behavior of MPTP-lesioned rats in the episodic-like memory test. (A) A schematic representation of the location of objects in the episodic-like memory test. This drawing shows an example of a possible arrangement of objects for the test in an open field. Animals received three trials—sample trial 1, sample trial 2, and the test trial (5 min each)—with 60 min intervals. In sample trials 1 and 2, four old objects (●) and four recent objects (◻) were presented to the animals, respectively. Two objects from sample trials 1 and 2, respectively, were placed at the same location as in the sample trials (Os and Rs). The remaining two objects were displaced to new locations (Od and Rd). (B) Sham + saline rats spent a longer time exploring the Os than the Od, and also a longer time exploring the Rd than the Rs. However, MPTP-lesioned animals (MPTP + saline) failed to recognize Rs and Rd. MPTP-treated rats receiving DCS treatment at the dosage of 10 mg/kg/day (MPTP + DCS 10), but not 5 mg/kg/day (MPTP + DCS 5), exhibited episodic-like memory behavior similar to that of Sham + saline rats. * $P < 0.05$, ** $P < 0.01$, # a trend of significance (see details in the text). Data are expressed as the mean \pm SEM.

detecting the effects of DCS on MPTP-induced neuroinflammation, neurodegeneration, and behavioral deficits in rats.

2. Materials and methods

2.1. Animals

The present study used 39 male Wistar rats (306.2 ± 0.9 g; BioLASCO Taiwan Co., Ltd.), housed in groups of five rats in acrylic cages ($35 \text{ cm} \times 56 \text{ cm} \times 19 \text{ cm}$) in an animal room with a 12-h light-dark cycle (lights on at 07:00 h) with food and water provided *ad libitum*. Each animal was handled for 5 min each day on three consecutive days prior to the experiment. Because research has demonstrated that high-intensity illumination is an aversive stimulus that decreases exploratory behavior in an unfamiliar environment [13], the present study performed all of its behavioral experiments under the dim illumination of red light (21 lx), as had been the case in our previous study [61,63]. The apparatus and objects used in this study were thoroughly cleaned with 20% ethanol before each rat was tested. All the 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 the Chung Shan Medical University (IACUC approval No.: 581).

2.2. Surgery and treatment

The animals were anesthetized by means of the intraperitoneal injection (i.p.) of 2 mg/kg of Zoletil 50 (Virbac, France). MPTP-HCl (Sigma, MO, USA) ($1 \mu\text{mol}$ in $2 \mu\text{l}$ of saline; 0.9% NaCl) was infused into the SNC through a 30-gauge stainless steel needle at a rate of $0.7 \mu\text{l}/\text{min}$ [7,14], and this infusion rested on the following coordinates adapted from the rat brain atlas [47]: anteroposterior (AP) -5.0 mm from the bregma, mediolateral (ML) ± 2.1 mm from the midline, and dorsoventral (DV) -7.7 mm from the skull. Sham-operated animals were submitted to the same procedures, where $2 \mu\text{l}$ saline instead of MPTP was infused bilaterally into the SNC. After surgery, the animals received a muscular injection of penicillin G-procaine (20,000 IU in 0.1 ml) to avoid infection and were left in observational boxes until they had recovered from anesthesia. Then, they were maintained in individual cages ($30 \text{ cm} \times 19 \text{ cm} \times 12 \text{ cm}$) for 6 days. Thereafter, they were returned to their home cages. During the first 5 postoperative days, in addition to the normal diet and water, all the animals were provided *ad libitum* a hypercaloric liquid diet (consisting of 20% sucrose) to prevent weight loss and to reduce the mortality caused by surgery [12]. DCS (Sigma, USA) was dissolved in saline immediately before usage and administered in a volume of 1 ml/kg of body weight [23]. Beginning 1 day after the surgery, MPTP-lesioned animals were treated daily with DCS (0, 5, or 10 mg/kg/day; i.p.) and assigned, accordingly, to MPTP + saline, MPTP + DCS 5, and MPTP + DCS 10 groups. Sham-operated animals were treated with saline (1 ml/kg/day; i.p.) (Sham + saline group).

2.3. Behavioral tests

2.3.1. Open field test

Seven days after the MPTP lesions were made, the rats underwent a motor function test (10 min) in an open field ($40 \text{ cm} \times 40 \text{ cm} \times 40 \text{ cm}$), in line with our previous report [22]. The following measures were recorded: (1) total distance: the expanse traveled in cm; (2) movement time; and (3) center time: the animal's center of body being within the center of the open field ($20 \text{ cm} \times 20 \text{ cm}$).

2.3.2. Episodic-like memory test

The episodic-like memory test was performed in another open field ($60 \text{ cm} \times 60 \text{ cm} \times 40 \text{ cm}$), 13 days after the MPTP lesion had been made. The floor of the open field was virtually divided into nine areas of equal size. A video camera was mounted 160 cm above the center of the open field to record behavior for off-line analysis. The open field had an open roof, so that the rats could perceive external distal cues.

We used two different objects (in quadruplicate) that were made of metal or plastic and that differed from each other in terms of base diameter, height, color, shape, and surface texture (the metal object: columnar in shape, 6.5 cm in diameter, 12 cm high, silver-colored, smooth-surfaced; the plastic object: cuboid in shape, a $7.5 \text{ cm} \times 5 \text{ cm}$ cross-section, 15 cm high, pellucid-colored, harsh-surfaced). Each of the two objects weighed about 950 g and was, therefore, unmovable by the rats. One day before the test, rats were placed individually into the open field and allowed to explore it twice (5 min each), with a 4 h interval, for adaptation to the testing environment [30].

The episodic-like memory test was consistent with previous reported research [37]. In brief, there were two sample trials and one testing trial. In the first sample trial, the rats were placed in the open field with four copies of objects (Fig. 1A, upper left), and were allowed to explore them for 5 min. After an interval of 60 min, the rats were put into the same open field again and underwent the second sample trial, which was identical to the first one except that a different set of objects was presented with a different spatial configuration (Fig. 1A, lower left). After an additional interval of 60 min, the rats underwent a testing trial, where two copies of the object from sample trial 1 (the "old" objects; "O") and two copies of the object from sample trial 2 (the "recent" objects; "R") were presented. In the testing trial, animals encountered a mixed set consisting of two "O" and two "R" objects. One object from each of the two sets of objects was placed in the "same" location as in the sample trials (Os and Rs). The other two objects were "displaced" to new locations (Od and Rd) (Fig. 1A, lower right). Time spent on exploring the objects was recorded.

2.4. Histological assay and image analysis

One day after the test of episodic-like memory, rats were sacrificed under deep anesthesia by means of CO_2 . For histological assessment, 5–6 randomly selected rats per group were perfused cardially with 4% paraformaldehyde in phosphate-buffered saline (PBS), and the brains were removed rapidly and post-fixed in 20% sucrose solution with 4% paraformaldehyde at 4°C until later 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

Table 1
Effects of D-cycloserine on behavior of MPTP-lesioned rats in the open field test.

	Sham	MPTP		
	DCS 0 mg/kg (n = 10)	DCS 0 mg/kg (n = 10)	DCS 5 mg/kg (n = 10)	DCS 10 mg/kg (n = 9)
Total distance (cm)	1758.6 ± 134.2	1509.8 ± 137.3	1486.4 ± 143.0	1556.6 ± 155.5
Movement time (s)	172.7 ± 18.6	148.8 ± 20.3	140.2 ± 23.6	172.9 ± 15.7
Center time (s)	268.4 ± 27.5	249.3 ± 27.5	238.4 ± 28.1	259.4 ± 27.8

DCS: D-cycloserine. Data are expressed as the mean ± SEM.

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 the one used in our previous report [61]. In sections containing the hippocampus, Nissl staining served to identify neurons therein.

The stained brain sections, identified according to the rat brain atlas [47], were used for measuring histological changes according to the methods described previously [61,64], and these measures took place by means of a microscope (ZEISS AXioskop2, Germany) coupled to a CCD (Optronics, USA) and the Image Pro Plus Software 6.0 (Media Cybernetics, CA, USA). In this study, we created three square areas of interest (measuring 36,477, 18,769, and 2354 μm^2) to determine the optical density of TH immunoreactivity in the striatum and the neuronal density in the SNc and hippocampal CA1 area, respectively. To measure the intensity of DAergic projections in the striatum, we converted the images of TH staining to gray-scale. The gray level of a given area of interest was measured, and the background staining, measured in non-immunoreactive corpus callosum, was subtracted. Thus, the relative optical density was restricted to the values generated by the TH reactive tissue. For measuring the density of DAergic neurons in the SNc, the images were captured but not converted to gray-scale, and an area of interest was overlaid in this region. The somas of TH immunoreactive neurons located in this area were counted. The density of activated microglia was measured, according to the methods described in the literature [56,61], in brain sections that were at equidistant positions. The number of activated microglial cells in the SNc, striatum, and hippocampus was counted in the areas of interest (measuring 18,769, 36,477, and 18,769 μm^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 μm -thick brain section. Thus, we represented the neuronal density by using a semi-quantitative method: calculating the percentage of an area occupied by Nissl-stained neurons in an area of interest in the CA1 area.

2.5. Data analysis

Behavior in the open field test was evaluated according to the one-way analysis of variance (ANOVA) followed by the least-significant difference post hoc test. Regarding the episodic-like memory test, we carried out—for each group

separately—additional 2×2 ANOVAs with two within-subject variables: object recency and location novelty. The tests could give us an overview regarding the main effects and the interaction of object recency and location novelty [37]. Since only comparisons between Os vs. Od and Rs vs. Rd within each group were crucial for evaluating behavior in the episodic-like memory test, a priori multiple *t*-tests followed by the Bonferroni post hoc test pertaining to the “exploring” time within each group were applied for object pairs: Os vs. Od and Rs vs. Rd. Each histological measurement was compared on the basis of one-way ANOVA followed by the least-significant difference post hoc test. All results are expressed as the mean ± SEM. The level of significance was defined as $P < 0.05$ (two-tailed).

3. Results

3.1. Open field test

One-way ANOVA revealed that the behavior in the open field—total distance ($F(3, 38) = 0.779$, $P = 0.514$), movement time ($F(3, 38) = 0.700$, $P = 0.558$), and center time ($F(3, 38) = 0.222$, $P = 0.881$)—was not different between the groups (Table 1).

3.2. Episodic-like memory test

One-way ANOVA showed that total object-exploration time in both sample trial 1 and sample trial 2 in the episodic-like memory test was not different between the groups (both P -values > 0.05 , data not shown). Similarly, total exploration time in the test trial in the Sham + saline (48.0 ± 8.2 s), MPTP + saline (58.8 ± 14.7 s), MPTP + DCS 5 (54.4 ± 5.9 s), and MPTP + DCS 10 (25.4 ± 4.3 s) groups exhibited no between-group differences ($F(3, 35) = 2.397$, $P = 0.085$), but did exhibit a tendency therein. The 2

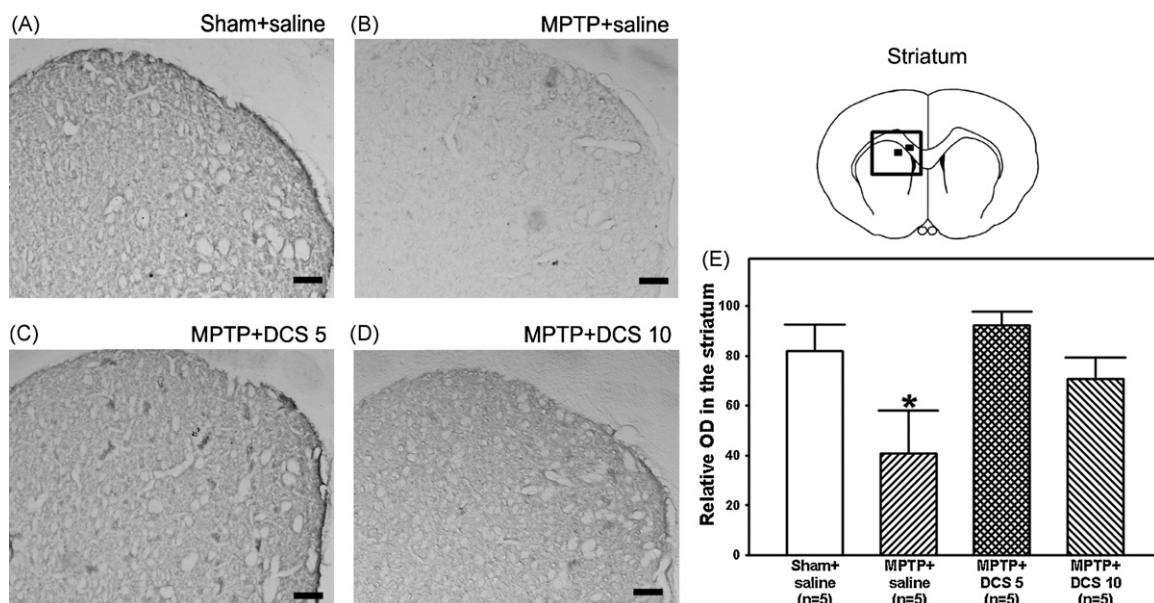


Fig. 2. Effects of D-cycloserine (DCS) on MPTP-induced changes of tyrosine hydroxylase (TH) immunoreactivity in the striatum. MPTP treatment (MPTP + saline) significantly decreased TH immunoreactivity in the striatum (B and E). DCS at the dosage of 5 mg/kg/day (MPTP + DCS 5) (C) and 10 mg/kg/day (MPTP + DCS 10) (D) reversed the MPTP-induced decrease in TH immunoreactivity. Magnification, 50 \times ; bar, 200 μm . Black squares in the schematic drawing are used for measuring optical density (OD). * $P < 0.05$, compared to Sham + saline group.

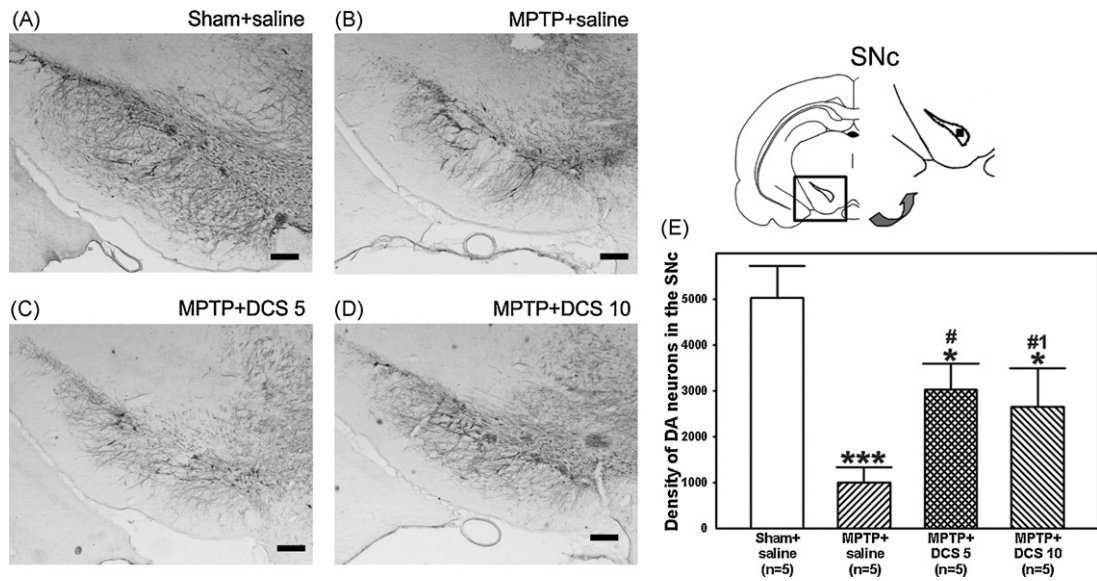


Fig. 3. Effects of D-cycloserine (DCS) on MPTP-induced changes in DAergic neurons in the SNc. DAergic neurons, stained by means of a tyrosine hydroxylase (TH) immunoreaction, are shown in the representative coronal sections. MPTP treatment (MPTP + saline) significantly decreased the density of DAergic neurons in the SNc (B and E). DCS at the dosage of 5 mg/kg/day (MPTP + DCS 5) (C) and 10 mg/kg/day (MPTP + DCS 10) (D) ameliorated the MPTP-induced decrease in the density of DAergic neurons. Magnification, 50 \times ; bar, 200 μ m. Black squares in the schematic drawings are used for measuring density of DAergic neurons. * P <0.05, *** P <0.001, compared to the Sham + saline group. # P <0.05, #1 P =0.081, compared to the MPTP + saline group.

(object recency) \times 2 (location) ANOVA with two within-subject factors was carried out for the four groups separately [37]. There were “object recency by location” interactions in the Sham + saline ($F(1, 9) = 13.747, P = 0.005$), MPTP + DCS 5 ($F(1, 9) = 5.795, P = 0.039$), and MPTP + DCS 10 ($F(1, 8) = 22.252, P = 0.002$) groups, respectively. However, no such interaction was found in the MPTP + saline group ($F(1, 9) = 1.516, P = 0.249$). A significant main effect of object recency was found in MPTP + saline rats ($F(1, 9) = 6.736, P = 0.029$) but not in other groups. Furthermore, no main effect of location was found in any group. A paired t -test revealed that rats in the Sham + saline (d.f. = 9, $t = 3.051, P < 0.05$), MPTP + saline (d.f. = 9, $t = 2.897, P < 0.05$), and MPTP + DCS 10 groups (d.f. = 8, $t = 3.249, P < 0.05$) spent a longer time exploring the old object placed in the same location (Os) than exploring the old object placed in a different location (Od). The time

spent on Os and Od in MPTP + DCS 5 rats had a trend of significance (d.f. = 9, $t = 2.101$, slightly less than the critical value 2.69) (Fig. 1B).

For the recent objects, a paired t -test revealed that rats in both the Sham + saline group (d.f. = 9, $t = -2.614$, slightly less than the critical value 2.69) and the MPTP + DCS 10 group (d.f. = 8, $t = -4.028, P < 0.001$) spent a longer time exploring the displaced object (Rd) than exploring the object located in the same place (Rs). However, no differences between exploring Rs and exploring Rd were found in either the MPTP + saline rats or the MPTP + DCS 5 rats (Fig. 1B).

3.3. Histology

Representative photomicrographs of immunostained and Nissl-stained brain sections are shown in Figs. 2–7. TH immunoreactivity

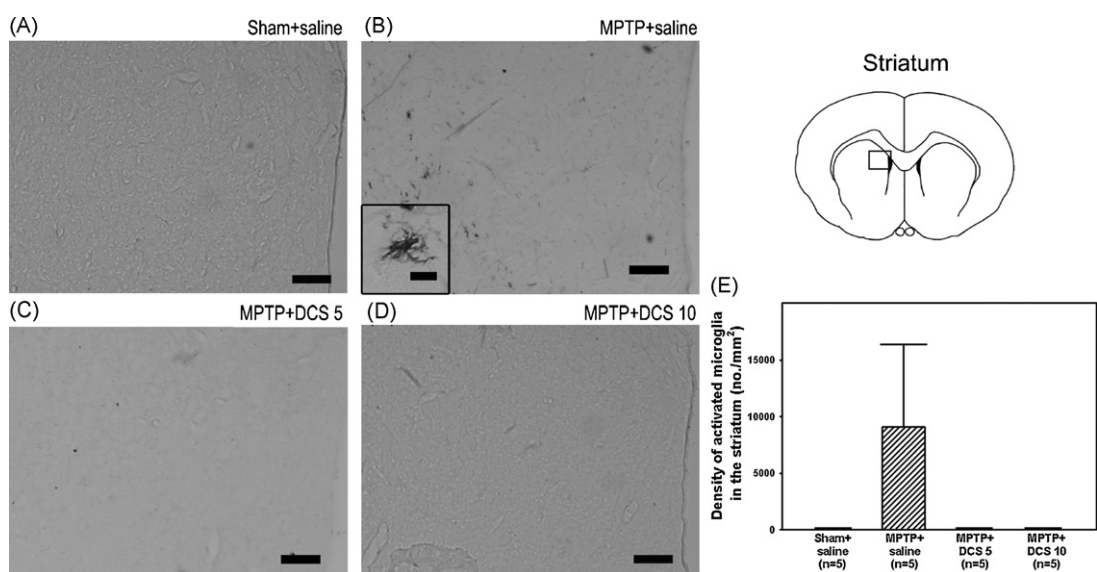


Fig. 4. Effects of D-cycloserine (DCS) on MPTP-induced activation of microglia in the striatum. Activated microglia, indicated as OX-6-positive cells, were observed in the striatum of the MPTP-lesioned group (MPTP + saline) (B). No activated microglia were found in the striatum of both the sham-operated control (Sham + saline) (A) and the MPTP-lesioned groups accompanied with DCS at 5 mg/kg/day (MPTP + DCS 5) (C) and 10 mg/kg/day (MPTP + DCS 10) (D). Magnification, 50 \times ; bar, 200 μ m. High magnification (200 \times ; bar, 20 μ m) of activated microglia is shown in the inset.

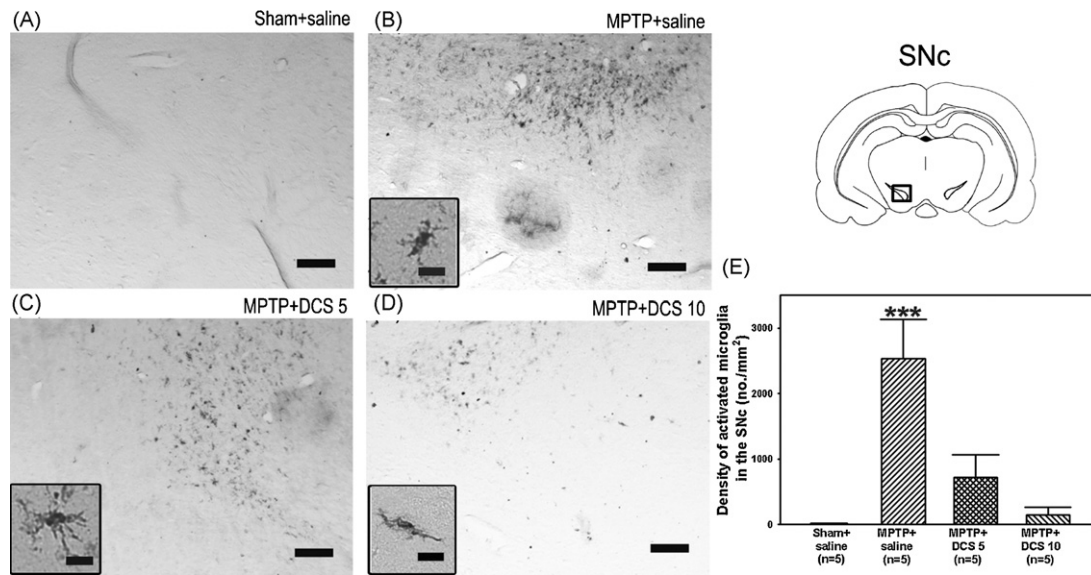


Fig. 5. Effects of D-cycloserine (DCS) on MPTP-induced activation of microglia in the SNc. No activated microglia, indicated as OX-6-positive cells, were found in the SNc of sham-operated (Sham + saline) rats (A). MPTP lesions (MPTP + saline) caused a massive accumulation of activated microglia in the SNc (B and E). DCS treatment at the dosage of 5 mg/kg/day (MPTP + DCS 5) (C) and 10 mg/kg/day (MPTP + DCS 10) (D) significantly decreased MPTP-induced microglial activation (E). Magnification, 50 \times ; bar, 200 μ m. High magnification (200 \times ; bar, 20 μ m) of activated microglia is shown in the insets. *** P < 0.001, compared to Sham + saline rats.

was observed in the cell body of DAergic neurons and the DAergic processes in the SNc and striatum, respectively. The resolution of the TH staining was sufficient for counting the cell number in an area of interest under light microscopy. Semi-quantitative analysis confirmed that rats in the MPTP + saline group exhibited both lowered relative optical density of TH immunoreactivity in the striatum ($P=0.020$) (Fig. 2B and E) and decreased density of DAergic neurons in the SNc ($P < 0.001$) (Fig. 3B and E), in comparison with the Sham + saline group. No differences in the optical density of the striatum's TH immunoreactivity were observed between the Sham + saline, MPTP + DCS 5, and MPTP + DCS 10 groups (Fig. 2C–E). In addition, the densities of DAergic neurons in the SNc of the MPTP + DCS 5 and MPTP + DCS 10 groups were significantly higher than the corresponding density in the MPTP + saline group ($P < 0.05$ and $P = 0.081$, respectively)

(Fig. 3C–E). Activated microglia, indicated by an accumulation of OX-6-positive cells, were detected in the striatum (Fig. 4), SNc (Fig. 5), and hippocampus (Fig. 6) of MPTP + saline rats. Semi-quantitative analysis showed that the density of activated microglia in the SNc (Fig. 5B and E) and hippocampus (Fig. 6B and E) of the MPTP + saline group was higher than in the Sham + saline controls (both P -values < 0.001). After the treatment of DCS, the density of activated microglia in the SNc (Fig. 5C–E) and hippocampus (Fig. 6C–E) of rats in the MPTP + DCS 5 and MPTP + DCS 10 groups was not different from that in the Sham + saline group. However, in the striatum, no significant difference in microglial activation was found between the groups (Fig. 4). In addition, the neuronal density in the pyramidal cell layer in the hippocampal CA1 area was decreased in the MPTP + saline group, compared with that in the Sham + saline group ($P < 0.001$). How-

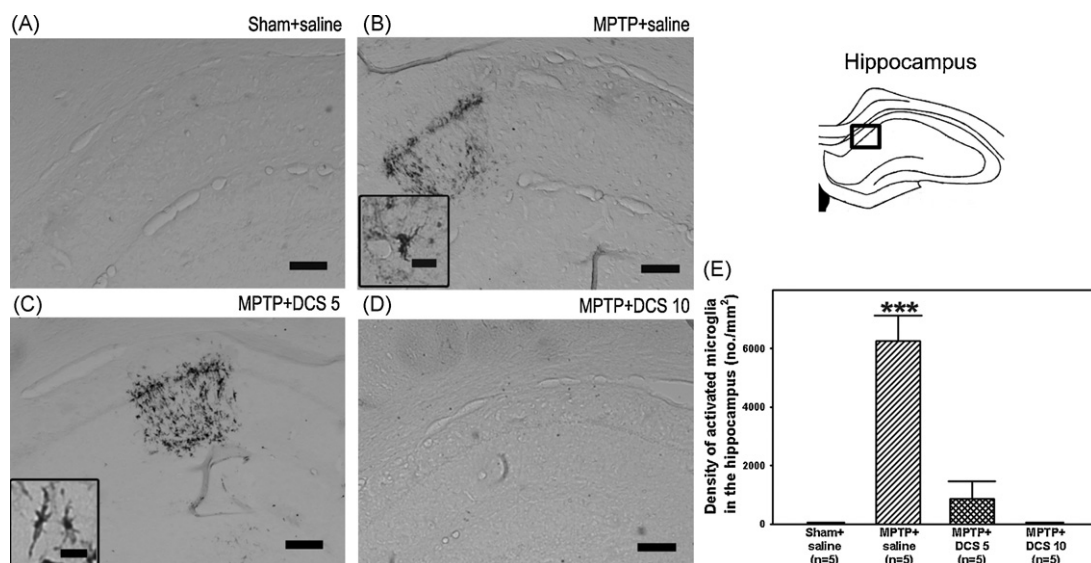


Fig. 6. Effects of D-cycloserine (DCS) on MPTP-induced activation of microglia in the hippocampus. No activated microglia, indicated as OX-6-positive cells, were found in the hippocampus of sham-operated (Sham + saline) rats (A). MPTP lesions (MPTP + saline) caused microglial activation in the hippocampus (B and E). DCS at the dosage of 5 mg/kg/day (MPTP + DCS 5) (C) and 10 mg/kg/day (MPTP + DCS 10) (D) suppressed MPTP-induced microglial activation (E). Magnification, 50 \times ; bar, 200 μ m. High magnification (200 \times ; bar, 20 μ m) of activated microglia is shown in the insets. *** P < 0.001, compared to the Sham + saline group.

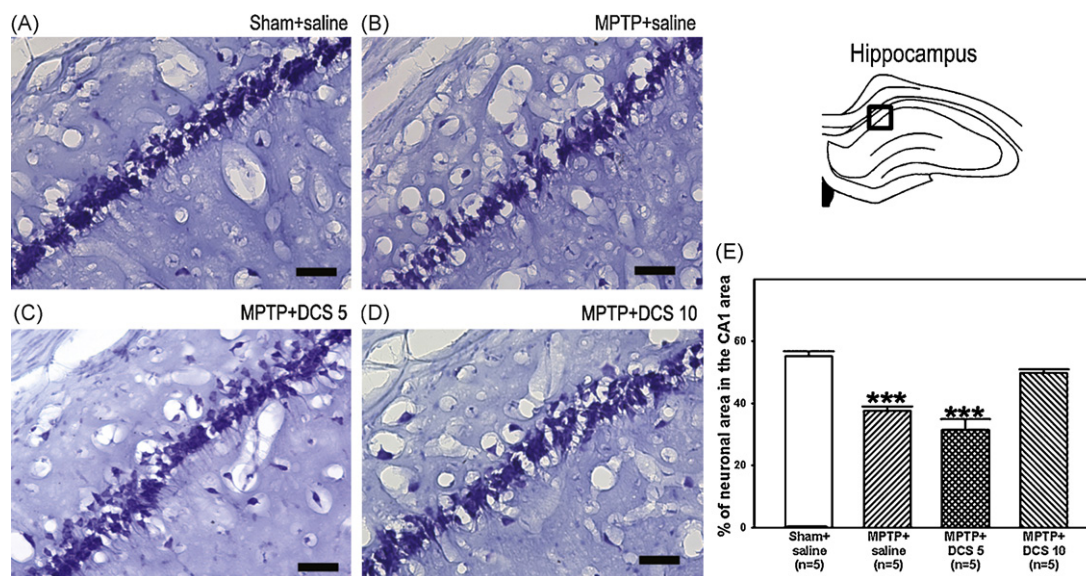


Fig. 7. Effects of D-cycloserine (DCS) on MPTP-induced cell loss in the hippocampal CA1 area. Images show Nissl-stained pyramidal neurons in the CA1 area of the hippocampus, as indicated in the square of the schematic drawing, in sham-operated rats (Sham + saline) (A) and MPTP-lesioned rats. MPTP lesions (MPTP + saline) (B) caused a decrease in neuronal density in the CA1 area, but the treatment of DCS at the dosage of 10 mg/kg/day (MPTP + DCS 10) (D) suppressed the above MPTP effect. Magnification, 200 \times ; bar, 100 μ m. *** P < 0.001, compared to the Sham + saline group.

ever, no such decrease was observed in the MPTP + DCS 10 group (Fig. 7).

4. Discussion

Seven days after the MPTP lesion, irrespective of DCS treatment, no significant changes in motor function and anxiety levels were found in the rats. Episodic-like memory was impaired 13 days after the MPTP lesions. DCS treatment at the dosage of 10 mg/kg/day reversed the above MPTP-induced deficits. Furthermore, MPTP lesions resulted in (1) DAergic degeneration in the SNc and striatum, (2) microglial activation in the SNc, striatum, and hippocampus, and (3) cell loss in the hippocampal CA1 area. DCS treatment (5 and 10 mg/kg/day) reversed not only MPTP-induced DAergic degeneration in the striatum and SNc, but also microglial activation in the SNc and hippocampus. Moreover, MPTP-induced cell loss in the hippocampal CA1 area was reversed by DCS at the dose of 10 mg/kg/day. These results suggest that DCS may act by modulating the NMDA receptor function to block MPTP-induced neuroinflammation, neurodegeneration, and deficits of episodic-like memory.

In line with previous studies showing transient motor dysfunction in the first week after MPTP lesions [5,12,59,61], the present study demonstrated no differences in total distance and center time in the open field test between sham-operated and MPTP-lesioned rats. Furthermore, DCS treatment did not affect open field behavior in MPTP-lesioned rats. Similarly, our previous study showed that DCS did not affect motor function in rats [23]. Because the center time in the open field test might reflect the anxiety level of rats [49], the present data indicate that motor function and anxiety level did not confound the behavior in the episodic-like memory test. However, there may have been moderate changes in either activity or motivation to explore the objects in the MPTP + DCS 10 group because the rats exhibited a lower total object-exploration time than did the sham-operated group.

The principle of the episodic-like memory test rests on the spontaneous exploratory behaviors of rats. The animals have a natural tendency to spend more time exploring novel than exploring familiar objects when there are two choices [18]. When the objects are familiar, the rats can remember the temporal order of their previ-

ous encounters and exhibit a preference for the older one [42]. In addition, the rats can discriminate according to objects' location; in the present study, the rats spent more time exploring the displaced objects [38]. Thus, regarding recent objects, rats indeed preferred one in a new location to one in the same location (i.e., rats spent a longer time exploring Rd than Rs). In contrast, for old objects, rats preferred one in the same location to one in a new location (i.e., rats spent a longer time exploring Os than Od), indicating that rats (1) can build an integrated memory system combining "what," "where," and "when" information and (2) can exhibit episodic-like memory behavior [30].

In the episodic-like memory test, the Os vs. Od and Rs vs. Rd comparisons reflect preference patterns based on the place changes for old and recent objects, respectively (Od and Rd were displaced to new locations). In agreement with previous studies [30,37], the present data reveal that sham-operated rats exhibited episodic-like memory behavior; that is, they spent a longer exploration time on Os and Rd than on Od and Rs, respectively, suggesting that these rats can recognize objects' location and objects' temporal order. MPTP lesions caused impairment in episodic-like memory. The spatial memory was disturbed in MPTP + saline rats because although these animals were able to recognize, as the controls did, place displacement of old objects (i.e., the rats spent a longer exploration time on Os than on Od), they were not able to recognize place displacement of recent objects (i.e., no exploration-time differences between Rs and Rd were observed). Further, MPTP-lesioned rats exhibited an exploration preference for the recent objects (i.e., rats explored recent objects more than old ones), and this preference may indicate disturbances in memory-recognition responses. Similar results have also been observed in MPTP-lesioned mice that exhibit impairments in habituation–dishabituation tasks [10] and decreases in preference for familiar foods [59]. In addition, the literature has indicated that MPTP causes cognitive deficits in animals, including learning impairment in the two-way active avoidance task [14] and disturbances in spatial navigation in the water maze test in rats [8,12,43], as well as a decrease in monkeys' recognition response during variable delayed-response tasks [54]. To our knowledge, the present data are the first to show the impairment of episodic-like memory in the MPTP-induced PD rat model. And we should note that, similarly, PD patients exhibited defective episodic

memory in a story-recall test, where they were not able to integrate “what,” “where,” and “when” elements in the stories [19].

In the present study, microglial activation, which is the massive accumulation of OX-6-positive cells [46], was observed in the striatum, SNc, and hippocampus, and the activation of microglia may take part in behavioral deficits in episodic-like memory tests. It has been demonstrated that a transient microglial reaction in the brain took place after the MPTP lesion, where the activated microglia were observed in the striatum of mice in the first post-lesion week [32,33]. In the current study, a significant increase in activated microglia was found in the SNc and hippocampus, as well as in the striatum (to a lesser extent) 2 weeks after MPTP lesions. In the striatum, there was a variance of microglial activation, which might reflect either the less robust response of glial cells in the striatum when DAergic degeneration occurs [41] or the disappearance of the transient activation of microglia in the striatum prior to removal of the brain [32]. Further, MPTP lesions induced a decrease in the density of pyramidal neurons in the hippocampal CA1 area. The hippocampus is involved in processing information essential for recognition memory concerning relative arrangements of items and may thus play an important role in episodic memory [60]. It has been demonstrated that hippocampal lesions in rats impair both spatial learning [15] and episodic-like memory [37]. Moreover, the hippocampal CA1 area is responsible for temporal memory [26]; lesions in this region correspond to—and likely account for—lower scores in the episodic-like memory test for rats [27]. In addition, microglial activation in the hippocampal CA1 area leads to learning and memory deficits in passive avoidance tests for rats [57]. Thus, both neuroinflammation, indicated by microglial activation, and cell loss in the hippocampal CA1 area may participate in the impairment of episodic-like memory in MPTP-lesioned rats. This point of view is supported by the fact that the recovery of episodic-like memory after DCS treatment was accompanied with suppression of MPTP-induced microglial activation and cell loss in the CA1 area.

MPTP treatment has been reported to elevate the release of glutamate in the striatum [53]. Overactivity of glutamatergic NMDA receptors results in excitotoxicity [44,48], which may participate in both cell loss in the DAergic system and microglial activation in the hippocampus of the PD brain [28]. The NMDA receptors play an important role in animals' cognitive functions. Blocking the NMDA receptors by MK-801 and phencyclidine has been reported to impair spatial learning in mice, where the animals fail to recognize objects whose locations have been changed [39]. Further, both (\pm)-3-amino-1-hydroxy-2-pyrrolidone (HA-966) and MK-801 depressed visual-recognition memory [40]. DCS, a partial agonist of the glycine binding site on the NMDA receptors [20], has been reported to facilitate responses in spatial recognition in the radial-arm maze test in MK-801-treated rats [31]. DCS has promnesic effects on rats' execution of learning tasks [25,36]. Furthermore, DCS improves memory in aging rats [2,3], reverses brain damage-induced impairments of long-term potentiation and object recognition [65], and enhances episodic-like memory in mice [66]. Researchers have observed glutamatergic hyperactivity in MPTP-lesioned mice, and have suggested that, therefore, glutamatergic hyperactivity is involved in microglial activation [44], excitotoxicity [48], and cognitive dysfunction [54]. Thus, DCS treatment may ameliorate neuroinflammation, cell loss, and behavioral deficits by regulating the activity of glutamatergic NMDA receptors. The choice of the dosage used in the present study was based on the fact that the effective dosage of DCS in behavioral studies ranges from 0.5 to 30 mg/kg [35]. In our previous study, the treatment of 5–30 mg/kg of DCS caused dose-related behavioral changes [23,63]. In the present study, 10 but not 5 mg/kg of DCS reversed MPTP-induced impairment of episodic-like memory.

Dementia in PD patient exhibit clinical and biochemical similarities to Alzheimer's disease (AD). A post-mortem study has

shown increased density of the NMDA receptors in the striatum and nucleus accumbens of both patients with PD and AD [58]. Memantine, an NMDA receptor antagonist, has been used to treat AD-related dementia. Amantadine is a precursor of memantine, also showing NMDA receptor-blocking activity. This drug was reported to be able to delay the onset of dementia in PD patients, and to attenuate the dementia's severity [29]. Because NMDA receptors play a crucial role in cognitive function, the application of NMDA receptor blockers to dementia cases is still controversial [50]. Treatment with DCS appeared to help ameliorate neuronal and behavioral functions in DAergic degeneration, providing evidence that there are connections between abnormality in glutamatergic system and dementia in PD. The activity of the partial agonist at the NMDA receptors may underlie the beneficial effects of DCS. However, it is not yet clear how DCS, exerting agonist activity on the NMDA receptors, can have beneficial effects on the conditions of glutamatergic hyperactivity where the NMDA receptors may have been over-activated. There are several possible explanations: (1) after a period of exposure (for example, 13 days in the present study), DCS may produce inhibitory rather than excitatory effects on the glutamatergic activity. This view may be supported by the findings that prolonged exposure to DCS causes desensitization of the NMDA receptors in cell cultures [4]. (2) The function of DCS may depend on the activity of the glutamatergic system [62]. For instance, DCS may exert its antagonist activity when the glutamatergic system is hyper-activated in the acute phase of MPTP lesions; however, DCS may exert its agonist activity in the chronic phase of MPTP lesion where the glutamatergic activity may have turned to hypo-activity. This point of view may be partially supported by our previous studies showing that behavioral and molecular effects of DCS depend on anxiety levels of rats [23,63]. Further, enhancing the efficacy of exposure psychotherapy by administration of DCS has been observed in patients with anxiety disorders [24,52] but not in non-clinical participants [16].

In summary, MPTP induced (1) DAergic degeneration, (2) microglial activation in the striatum, SNc, and hippocampus, (3) pyramidal cell loss in the hippocampal CA1 area, and (4) impairment of episodic-like memory. DCS treatment at the dosage of 10 mg/kg/day ameliorated neuroinflammation in the brain, reversed the density of CA1 pyramidal neurons, and restored the performance of episodic-like memory in MPTP-lesioned rats. These results suggest that NMDA receptors may be involved in cognitive deficits in PD. Moreover, because it is possible to induce a pure episodic-like memory deficit in rats without impairing the what, where, and when memory per se [37], the episodic-like memory system may be more sensitive to manipulation than the “three Ws” memory themselves. As a consequence, a treatment perhaps can restore a PD patient's “three Ws” memory function while failing to restore the patient's episodic memory. In this regard, the current findings that DCS can recover animals from episodic-like memory deficits induced by MPTP are of immense significance. Since DCS is already in use in humans both for several indications and with a good safety profile [11,21,34,45] and is able to improve cognitive function in human psychotherapy [24], its application in the treatment of PD-related dementia is warranted.

Acknowledgments

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