行政院國家科學委員會專題研究計畫 成果報告

探討麩胺酸神經系統在 MPTP 所誘發之神經發炎及行為缺陷的角色：嘗試建立巴金森氏症失智的動物模式
研究成果報告(精簡版)

計 畫 類 別 ：個別型
計 畫 編 號 ：NSC 98-2410-H-040-003-
執 行 期 間 ：98 年 08 月 01 日至 99 年 07 月 31 日
執 行 單 位 ：中山醫學大學心理學系（所）（臨床組）

計 畫 主 持 人 ：何應瑞

報 告 附 件 ：出席國際會議研究心得報告及發表論文

處 理 方 式 ：本計畫可公開查詢

中 華 民 國 98 年 09 月 30 日

行政院國家科學委員會專題研究計畫

探討麩胺酸神經系統在 MPTP 所誘發之神經發炎及行為缺陷的角色：嘗試建立巴金森氏症失智的動物模式
研究成果報告(精簡版)

計畫類別： 個別型

計畫編號： NSC98-2410-H-040-003

執行期間： 98 年 08 月 01 日至 99 年 07 月 31 日

執行單位： 中山醫學大學心理學系（所）（臨床組）

計畫主持人： 何應瑞

備註： 本計畫可公開查詢

中 華 民 國 98 年 09 月 30 日

MPTP-induced dopaminergic degeneration and deficits in object recognition are accompanied with neuroinflammation in the hippocampus

Hiu-Ngar Sy[1#], Shey-Lin Wu[1, 2#], Wen-Fu Wang[1], An-Li Wang[3], Yao-Ting Huang[1], Ying-Ming Liou[3], Cornelius Rainer Pawlak[4*], Ying-Jui Ho[5*]

[1] Department of Neurology, Chang-Hua Christian Hospital, Taiwan, ROC

[2] Department of Bioindustry Technology, Dayeh University, Taiwan, ROC

[3] Department of Life Sciences, National Chung Hsing University, Taiwan, ROC

[4] Institute of Neuropsychology and Clinical Psychology, Central Institute of Mental Health, Mannheim, Germany

[5] School of Psychology, Chung Shan Medical University, Taiwan, ROC

[#]equal contribution

***Corresponding authors:**

Ying-Jui Ho Ph.D.

School of Psychology, Chung Shan Medical University, Taiwan, ROC

Address: No. 110, Sec. 1, Jianguo N. Rd., Taichung City 402, Taiwan, ROC

E-mail: yjho@csmu.edu.tw; joshuayjho@yahoo.com.tw

Tel: +886-4-24730022 ext. 11858; Fax: +886-4-23248191

Cornelius Rainer Pawlak Ph.D.

Institute of Neuropsychology and Clinical Psychology, Central Institute of Mental Health, Mannheim, Germany

Address: J5, D-68159 Mannheim, Germany

E-mail: cornelius.pawlak@zi-mannheim.de

Tel: +49 (0) 621 1703 6262; Fax: +49 (0) 621 1703 6255

Abstract

Emotional changes, impairment on object recognition, and neuroinflammation are seen in Parkinson's disease with dementia (PDD). Here, we showed that bilateral infusion of 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) into the rat substantia nigra pars compacta (SNc) of Wistar rats caused degeneration of nigrostriatal dopaminergic neurons, microglial activation in the SNc and hippocampus, as well as cell loss in the hippocampal CA1 area. With regard to behavior, increase of anxiety-like behavior and impairment of object recognition were observed during the fourth week after the MPTP lesion. The behavioral changes were not caused by motor impairment, since the rats had already recovered from MPTP-induced catalepsy before the tests were performed. These findings show that MPTP-induced neuroinflammation and its consequences, for example, microglial activation and cell loss in the hippocampus, may contribute to dopaminergic degeneration-related behavioral deficits and suggest that, in addition to the dopaminergic system, the limbic system may also participate in the pathophysiology of PDD.

Keywords: Parkinson's disease, dementia, object recognition, neuroinflammation, microglial activation, MPTP

1. Introduction

Parkinson's disease (PD) is a high prevalent neurodegenerative disorder (Aarsland et al., 2005). In addition to the motor dysfunctions, cognitive impairment and dementia are seen in a high percentage of PD patients (Brown and Marsden, 1984; Owen et al., 1995). The proportion of PD patients with dementia is 25-30%, an incidence up to six times higher than that in healthy people (Aarsland et al., 2001). Emotional changes (Levin et al., 1991), for example elevation of anxiety levels (Fenelon et al., 2000), visuospatial dysfunctions (Crucian and Okun, 2003; Emre, 2003), as well as impairments on facial recognition and object perception (Barnes et al., 2003; Laatu et al., 2004; Ramirez-Ruiz et al., 2006) are the main symptoms in Parkinson's disease dementia (PDD).

Although the loss of dopamine (DA)-containing neurons originated in the substantia nigra is the main character of PD (McGeer and McGeer, 2004), inflammation has also been proposed as a possible mechanism in the pathogenesis of PD, as the intensity of inflammation in the substantia nigra is considerably higher in PD patients than in controls (Abramsky and Litvin, 1978; McGeer and McGeer, 2004). Nigrostriatal dysfunction alone is probably not sufficient for the development of dementia in PD because activated microglia have been observed not only in the substantia nigra and putamen, where DA loss is prominent, but also in the hippocampus of PD patients (McGeer et al., 1988; McGeer and McGeer, 1995; Sawada et al., 2006). Microglial activation in the hippocampus has been suggested to be responsible for functional changes in neurons and cognitive decline in PD and dementia with Lewy bodies (Imamura et al., 2005). Emotional changes and cognitive disturbances, particularly in tests involving spatial learning and memory, are linked with aberrations in the

mesocorticolimbic and striatal systems (Caraceni et al., 1993; Denicoff et al., 1987; West et al., 1987). Thus, microglial activation in these areas may also be involved in the pathophysiology of PDD.

Literatures have reported deficits in habit learning and spatial memory in the active avoidance test (Da Cunha et al., 2001) and in water maze test (Da Cunha et al., 2002; Da Cunha et al., 2003), respectively, in MPTP-induced rat PD model. Disturbance in visual discrimination in MPTP-treated monkeys has also been indicated (Schneider et al., 2000). However, no data are available in object recognition in MPTP-treated rodents, which would better model the disability of object recognition in PDD. To better understand the pathophysiology of PDD, it is necessary to detect neuronal changes occur after degeneration of the nigrostriatal DAergic system. Animals treated with 1-methyl-4-phenyl-1,2,3,6-terahydropyriding (MPTP) are widely used as a PD animal model, as MPTP selectively destroys the DAergic system and causes microglial activation in humans (Mogi et al., 1996). Impairment of executive and visuospatial functions is observed not only in patients with PDD (Crucian and Okun, 2003; Emre, 2003) but also in people exposed to MPTP (Stern et al., 1990). However, it is not known whether the MPTP-induced inflammation and behavioral changes seen in animal studies resemble the pathophysiology and symptoms seen in PDD. We examined motor and emotional behavior as well as object recognition in rats after MPTP lesion using a battery of behavioral tests, namely a bar test as a measure of catalepsy (Sanberg et al., 1988), an elevated plus-maze as a measure of anxiety-like behavior (Ho et al., 2002), and an object recognition test in an open field (Mumby et al., 2005). In addition, we analyzed microglial activation and cell loss in the brain. The results showed that MPTP-induced DAergic degeneration and deficits in emotional behavior and object

recognition were accompanied with neuroinflammation in the hippocampus. It is thus suggested that, in addition to the DAergic system, the limbic system may also participate in the pathophysiology of PDD.

2. Methods

2.1 Animals

Male Wistar rats (384 ± 4.5 g; n = 40; National Laboratory Animal Center, ROC) were housed in groups of five in acrylic cages (35 × 56 × 19 cm) in an animal room with a 12 h light-dark cycle (lights on at 07:00 h) 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 the experimental procedures were performed according to the NIH Guide for Care and Use of Laboratory Animals and were approved by the Animal Care Committee of Chung Shan Medical University (IACUC approval NO: 699).

2.2 General procedures

All animals underwent stereotaxic surgery and bilateral infusion into the substantia nigra pars compacta (SNc) of either MPTP-HCl (1 μmol in 2 μl of saline; Sigma, USA) or vehicle (day 0) (see surgery section below). They were then subjected to a bar test on days 3, 5, 7, and 9 and an elevated plus-maze test on day 25, followed by an object recognition test starting on day 25 and finishing on day 27, all of which were started at least 2 h after the beginning of the light-phase (7:00 h). Behavioral tests were performed in a dim observation room (28 lux red light). The test equipment and object were cleaned using 20% ethanol and thoroughly dried before each test trial. On day 28 after MPTP treatment, the rats were sacrificed by exposure to CO_2, transcardially perfused with phosphate-buffered saline (PBS) and followed by 4% paraformaldehyde

in PBS. The brains were immediately removed and post-fixed in 20% sucrose solution with 4% paraformaldehyde at 4°C for histochemical assessment.

2.3. Surgery

Brain surgery was performed on a stereotaxic instrument. The rats received intraperitoneal injection (IP) of atropine sulfate (0.4 mg/kg) to suppress salivation and were anesthetized using Zoletil (2 mg/kg, IP; Virbac, Carros, France). In the lesion group (n = 25), MPTP-HCl (1 μmol in 2 μl of saline) was bilaterally infused into the SNc through a 30 gauge stainless needle at a rate of 0.7 μl/min at a site with the following coordinates adapted from the rat brain atlas (Paxinos and Watson, 1986): AP -5.0 mm, ML ± 1.8 mm, DV -8.0 mm from the bregma. Sham-operated rats were subjected to the same procedure, but were infused with 2 μl of saline instead of MPTP (n = 15). Immediately after surgery, the rats received a muscular injection with penicillin-G procaine (0.2 ml, 20,000 IU) and housed individually in plastic cages (25 × 41 × 19 cm) for 10 days, then 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 (Da Cunha et al., 2001; Ferro et al., 2005).

2.4. Behavioral tests

Behavior in the bar test was scored manually by a trained observer blind to the treatment conditions. The elevated plus-maze and object recognition test was monitored and scored by using a video camera positioned above the apparatus and a home-made video image analysis system (VIAS) (Li and Chao, 2008). The spatial resolution of the VIAS was set to 0.7 cm; and the image processing capability was higher than 14 pictures/sec.

Bar test: The bar test was performed on days 3, 5, 7, and 9 after MPTP lesion. Catalepsy was measured as the mean time spent by a rat to climb over a 9 cm high bar after being laid across it with its hind limbs on the floor (Sanberg et al., 1988). Each animal was tested in 3 consecutive trials on each trial day.

Elevated plus-maze test: Unconditioned anxiety-like avoidance behavior was assessed as our previous report, using the elevated plus-maze test (Ho et al., 2005), performed on day 25. The measures recorded were: (1) open arm latency, that is, the time from placing the rat into the plus-maze until it entered one of the open arms, (2) the time spent on and (3) the number of entries into open or enclosed arms, (4) total distance, that is, the distance traveled by the rat in cm, and (5) rearing number.

Object recognition test: The apparatus and testing procedure for the object recognition test were similar to those described previously (Mumby et al., 2005). An open field arena was constructed of black polyvinyl plastic (100 cm long × 100 cm wide × 60 cm high). Each rat was subjected to 3 exposure sessions at 24 h intervals. Five minutes after the last exposure session, a test trial was performed. Four different objects made of transparent glass, paper, porcelain, or metal (all sizes around 10 × 10 × 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. On day 25 (5 min after the elevated plus-maze test), the rat was placed in the only free corner and allowed to explore the objects for a 5 min exposure session, and also on the next 2 consecutive days. Five minutes after the last exposure session, object “B” was replaced by a novel object “D” and the animal was returned to the open field for a 5 min test session. The time spent exploring the objects and the number of rearing during the exposure and test sessions were recorded. The percentage

of the exploration time spent on object B or D in the sessions [(Time $_{B \text{ or } D}$ / Time $_{all\ objects}$) × 100%] was calculated. The percentage of time spent exploring the novel object "D" served as the measure of recognition memory for the familiar object. Exploration of an object was defined as the rat approaching an object and having physical contact with it, either with its snout and/or forepaws.

2.5. Histological assay

To detect DAergic degeneration and microglial activation, frozen coronal brain sections (30 μm) were cut, rinsed in PBS, picked up on gelatinized slides, and immunostained at 4°C overnight with mouse monoclonal antibodies against rat tyrosine hydroxylase (TH) (1:4 000; Zymade, USA) or rat MHC class II (OX-6; 1:400; BD Biosciences Pharmingen, CA, USA) diluted in PBS. OX-6 selectively stains activated microglia (Ogura et al., 1994). The sections were incubated sequentially for 30 min at 37ºC with biotinylated horse anti-mouse IgG antibody (Vector Laboratory, CA, USA) and avidin-biotin-horseradish peroxidase complex (ABC Elite HRP kit; Vector Laboratory, CA, USA), then were incubated for 30 min at room temperature with 0.02% 3,3'-diaminobenzidine (Sigma, USA). The reaction was stopped by extensive washing with PBS. To detect cell loss, Nissl staining was used to identify neurons.

Image analysis: The stained brain sections, identified according to the rat brain atlas (Paxinos and Watson, 1986), were used for measuring histological changes with the methods described previously (Xavier et al., 2005), using a microscope (ZEISS AXioskop2, Germany) coupled to a CCD (Optronics, USA) and the Image Pro Plus Software 4.1 (Media Cybernetics, CA, USA). In this study, an area of interest were created, measuring 24 585 μm^2, to determine the optical density of TH immunoreactivity in the striatum and neuronal density in the SNc, and 40 000 μm^2 for

counting the neuronal density in the hippocampal CA1 area. To measure the intensity of DAergic projections in the striatum, the images of TH staining were converted to gray scale. The gray level of 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. To measure the density of DAergic neurons in the SNc, the images were captured, 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. Because the neurons are tightly packed, it was difficult to directly calculate the number of pyramidal neurons in the CA1 area from a 30 μm-thick brain section. Thus, a semi-quantitative method, percentage of area of Nissl-stained neurons in an area of interest in the CA1 area, was used to represent the neuronal density.

2.6. Data analysis

Statistical testing was performed to compare the two groups using the *t*-test. Analysis of variance (ANOVA) for repeated measures, followed by Scheffé's post hoc test, and evaluation of behavioral correlations were used when appropriate. All results are expressed as the mean ± SEM. The level of significance was defined as $P < 0.05$ (two-tailed).

3. Results

Behavior: After MPTP lesion, the animals showed a significant and transitory catalepsy, i.e., an increase in the crossing latency in the bar test. ANOVA with repeated measures revealed significant main effects of time ($F(3,114) = 7.03$, $P < 0.001$) and lesion ($F(1,38) = 10.05$, $P < 0.01$) as well as time×lesion interaction ($F(3,114) = 6.66$, P

< 0.001). Significant longer crossing latencies in the MPTP-treated animals were seen on days 3 and 5 after MPTP lesion (d.f. = 38, *t*–values ≧ 2.54, *P*-values < 0.05), while, on days 7 and 9, MPTP- and sham-treated rats displayed comparable crossing latencies (Fig. 1.).

【Figure 1 should be here】

An increased open arm latency, decreased open arm time, and less number of open arm entries in the elevated plus-maze test, compared to the controls, were seen in MPTP-treated rats on day 25 (d.f. = 38, *t*-values > 1.98, *P*-values < 0.05), indicating higher anxiety-like levels. However, the number of rearing, enclosed arm entries, and total distance traveled by the rats in the elevated plus-maze test, which are commonly used to measure general activity, were not different between the groups (Table 1).

【Table 1 should be here】

MPTP-treated rats spent a lower percentage of time exploring the novel object "D" on day 27 after MPTP lesion than the sham-operated controls (d.f. = 38, $t = 2.94$, $P < 0.01$) (Fig. 2A). As shown in Fig. 2B, ANOVA with repeated measures revealed a significant time effect ($F(1,114) = 24.86$, $P < 0.001$) for rearing number, but no significant lesion effect or time×lesion interaction.

【Figure 2 should be here】

The open arm time and number of open arm entries in the elevated plus-maze test were significantly correlated with percentage of time spent on exploring novel object (*P*-values < 0.05). However, enclosed arm time in the elevated plus-maze test was negatively correlated with percentage of time spent on exploring novel object and the number of rearing in the object recognition test (*P*-values < 0.05) (Tab. 2.).

【Table 2 should be here】

Histology: Representative photomicrographs of immunostained and Nissl-stained brain sections are shown in Figs. 3-5. TH positive neurons were found in the striatum and SNc of the brain from sham-operated and MPTP-treated groups. TH immunoreactivity was observed in neuronal cell bodies and their processes. MPTP treatment induced a reduction in relative optical density of TH immunoreactivity in the striatum (df = 14, t = 3.71, $P < 0.01$) and a decrease in density of DAergic neurons in the SNc (df = 14, t = 2.62, $P < 0.05$), compared to the sham-operated group (Fig. 3). Microglial activation, indicated by an accumulation of OX-6-positive cells, was evident in the SNc and hippocampus (Fig. 4), but not in the prefrontal cortex, striatum, or cerebral cortex (data not shown). Semi-quantitative analysis confirmed that MPTP lesion decreased the neuronal density in the pyramidal cell layer in the hippocampal CA1 area (df = 14, t = 4.92, $P < 0.001$), compared to the sham-operated rats (Fig. 5).

【Figure 3-5 should be here】

4. Discussion

Four weeks after intra-SNc infusion of MPTP, there were neurodegeneration of the nigrostriatal DAergic system, cell loss in the hippocampal CA1 area, as well as microglial activation in the SNc and hippocampus. Furthermore, emotional-related and cognitive changes were also observed during the fourth week after MPTP lesion, namely, increased anxiety-like avoidance behavior and impairment of object recognition. We did not find evidence of motor impairment 7 days after MPTP lesion when catalepsy was no longer observed, suggesting that the behavioral differences seen in subsequent tests were not due to motor deterioration. These results suggest that the MPTP-induced neuroinflammation and cell loss in the hippocampus may contribute to

DAergic degeneration-related behavioral deficits.

MPTP is a toxin commonly used to induce PD animal model because it causes specific degeneration of DAergic neurons in the substantia nigra and the loss of nerve terminals in the striatum. In the current study, intra-SNc infusion of MPTP resulted in a significant decrease in TH immunohistochemical staining in the SNc and striatum, indicating DAergic lesion. Although calculating the cell number in the representative brain sections yielded similar histological results reported in the literature, stereologic approach by counting cells in a complete series of sections can provide additional data (Ferro et al., 2005; Meissner et al., 2003). MPTP-lesioned rats have been reported showing lower than controls in spontaneous locomotion in the open field test one day after the surgery (Capitelli et al., 2008; Perry et al., 2005; Reksidler et al., 2008). However, the behavioral changes were no longer observed 7 (Capitelli et al., 2008) and 18 days (Ferro et al., 2005; Perry et al., 2005) after the lesion. Further, in line with previous reports showing transitory catalepsy by using the bar test (Ferro et al., 2005; Sedelis et al., 2001), catalepsy was seen during the first 5 days, but not on days 7 and 9 after MPTP lesion. Recovery of motor function was further supported by the lack of differences between the groups in the rearing number, enclosed arm entries, and distance traveled by the rats in the elevated plus-maze test, as well as rearing number in the object recognition test, indicating absence of gross motor impairment and suggesting that the behavioral performance in the tests was not confounded by motor impairment.

In consistent with the fact that high anxiety levels have been indicated in the late course of PD (Fenelon et al., 2000), MPTP lesion caused an enhancement of anxiety-like behavior in the elevated plus-maze test, indicated as lowered open arm time

and increased enclosed arm time. In addition, dysfunction of object recognition in animal model may be compatible with some phenomenon of visuospatial (Girotti et al., 1988), facial recognition, and object perception deficits (Barnes et al., 2003; Ramirez-Ruiz et al., 2006) in PD patients. Interestingly, anxiety levels were negatively correlated with the time spent exploring novel object but not correlated with total exploration time. This result was consistent with the findings that cognitive despaired PD patients show co-existence of anxiety disorders (Fenelon et al., 2000). It has been reported that cognitively deteriorated PD patients performed more poorly than the healthy controls and cognitively preserved PD patients in discriminating objects, where the subjects used visual sensation for the object recognition task because they need to response to the stimuli, pictures or written words, presented on a screen (Laatu et al., 2004). It is noteworthy that the animals in the current study may not totally rely on visual sensation to recognize the objects. Further, it has been demonstrated that MPTP-treated rats show deficits in performing a spatial working memory in the Morris water maze task (Ferro et al., 2005), and in acquisition and retention processes in an active avoidance test (Da Cunha et al., 2001), suggesting that MPTP-treated rats may be a model for early Parkinson's disease amnesia. The hippocampus is important for spatial navigation (Zhang et al., 2004), recognition memory (Broadbent et al., 2004), and short-term memory associating objects and their locations (Li and Chao, 2008; Piekema et al., 2006). Moreover, the hippocampal CA1 region plays a critical role in object recognition because ischemia-induced cell loss in the hippocampal CA1 area of rats produced object recognition deficits in the acquisition and retention in delayed nonmatching-to-sample task (Wood et al., 1993). Furthermore, local pharmacological manipulations in the CA1 area are also affecting consolidation of object recognition

memory (Clarke et al., 2008; de Lima et al., 2006). In agree with the above findings, our present data showing that MPTP lesion causes inflammation and cell loss in the hippocampal CA1 pyramidal neurons may provide possible neuronal mechanisms underling the MPTP-induced object recognition impairment and may prove to be a useful tool in assessing the ability of pharmacological agents to prevent neurodegeneration-related recognition deficits.

Immunohistochemical alterations in the brain have been suggested to be involved the behavioral changes seen after DAergic degeneration (Kurosaki et al., 2004). Although DAergic deficit is the main neurochemical impairment in PD, clinical data show that dementia in PD patients does not improve with levodopa treatment (Lewis et al., 2005) and that motor symptoms and cognitive dysfunction in PDD patients are correlated strongly with non-DAergic systems (Levy et al., 2002). Microglia, the resident immune cells of the central nervous system, act as regulators of the secretion of neurotrophic and neurotoxic factors (Kadiu et al., 2005). Sawada et al. have proposed that activated microglia may change *in vivo* from neuroprotective to neurotoxic subsets as degeneration of DAergic neurons in the substantia nigra progresses in PD (Sawada et al., 2006). Chronic activation of microglia causes inflammatory responses and may, through the release of cytokines, lead to neuronal damage (Dheen et al., 2007; Imamura et al., 2003). Microglial activation and increased levels of inflammatory cytokines have been observed in the substantia nigra, putamen, and hippocampus in PD patients (McGeer et al., 1988; McGeer and McGeer, 1995; Sawada et al., 2006). Furthermore, neuroinflammation has been suggested to be responsible for neuronal degeneration and cognitive decline in PD and dementia with Lewy bodies (Imamura et al., 2005). Similarly, in the present study, MPTP caused a chronic activation of microglia in the

SNc and hippocampus, which may participate in the MPTP-induced emotional and cognitive deficits, notwithstanding the fact that other factors are probably also involved. The mechanisms by which degeneration of DAergic neurons causes neuroinflammation in the limbic system need to be studied further. In addition, non-steroidal anti-inflammatory drugs are able to reduce neuronal death induced by activated microglia and improve the motor impairment in PD (Hirohata et al., 2008; Klegeris and McGeer, 2005). Thus, suppression of microglial activation by using anti-inflammatory compounds may therefore be a feasible strategy for preventing neuronal and cognitive decline in PD (Sriram et al., 2006).

In addition to the DAergic degeneration in the SNc and striatum, consistent with the pathophysiology of PD, intra-nigral infusion of MPTP also caused increases in microglial activation in the brain, cell loss in the hippocampal CA1 area, emotional changes, and object recognition deficits. These findings show that MPTP-induced neuroinflammation and its consequences may contribute to DAergic degeneration-related behavioral deficits and suggest that, in addition to the DAergic system, the limbic system may also participate in the pathophysiology of PDD.

Acknowledgments

The authors thank Drs. Yuan-Feen Tsai and Rainer K. Schwarting for their thoughtful comments on this manuscript, Dr. Jay-Shake Li and Mr. Sheng-Yuan Wang for kindly providing their self-developed software to collect the behavioral data, and also Ms. Shih-Chun Ho for her technical assistance. This work was supported by grants from the National Science Council of the ROC (NSC 96-2320-B-040-019; DAAD/NSC

Project Based Personnel Exchange Program 0950042882P), Chang-Hua Christian Hospital (CCH grant 93118 and 92137), Chung Shan Medical University (097-CCH-CSMU-14), and German Research Foundation (DFG PA 818/4-1).

References

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;56:730-736.

Aarsland, D, Zaccai, J, Brayne, C. A systematic review of prevalence studies of dementia in Parkinson's disease. Mov Disord 2005;20:1255-1263.

Abramsky, O, Litvin, Y. Automimmune response to dopamine-receptor as a possible mechanism in the pathogenesis of Parkinson's disease and schizophrenia. Perspect Biol Med 1978;22:104-114.

Barnes, J, Boubert, L, Harris, J, Lee, A, David, AS. Reality monitoring and visual hallucinations in Parkinson's disease. Neuropsychologia 2003;41:565-574.

Broadbent, NJ, Squire, LR, Clark, RE. Spatial memory, recognition memory, and the hippocampus. Proc Natl Acad Sci U S A 2004;101:14515-14520.

Brown, RG, Marsden, CD. How common is dementia in Parkinson's disease? Lancet 1984;2:1262-1265.

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;594:101-108.

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:1266-1269.

Clarke, JR, Rossato, JI, Monteiro, S, Bevilaqua, LR, Izquierdo, I, Cammarota, M. Posttraining activation of CB1 cannabinoid receptors in the CA1 region of the

dorsal hippocampus impairs object recognition long-term memory. Neurobiol Learn Mem 2008;90:374-381.

Crucian, GP, Okun, MS. Visual-spatial ability in Parkinson's disease. Front Biosci 2003;8:s992-997.

Da Cunha, C, Angelucci, ME, Canteras, NS, Wonnacott, S, Takahashi, RN. The lesion of the rat substantia nigra pars compacta dopaminergic neurons as a model for Parkinson's disease memory disabilities. Cell Mol Neurobiol 2002;22:227-237.

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.

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;79:236-242.

de Lima, MN, Luft, T, Roesler, R, Schroder, N. Temporary inactivation reveals an essential role of the dorsal hippocampus in consolidation of object recognition memory. Neurosci Lett 2006;405:142-146.

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.

Dheen, ST, Kaur, C, Ling, EA. Microglial activation and its implications in the brain diseases. Curr Med Chem 2007;14:1189-1197.

Emre, M. Dementia associated with Parkinson's disease. Lancet Neurol 2003;2:229-237.

Fenelon, G, Mahieux, F, Huon, R, Ziegler, M. Hallucinations in Parkinson's disease:

prevalence, phenomenology and risk factors. Brain 2000;123 (Pt 4):733-745.

Ferro, MM, Bellissimo, MI, Anselmo-Franci, JA, Angellucci, 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.

Girotti, F, Soliveri, P, Carella, F, Piccolo, I, Caffarra, P, Musicco, M, et al. Dementia and cognitive impairment in Parkinson's disease. J Neurol Neurosur Ps 1988;51:1498-1502.

Hirohata, M, Ono, K, Morinaga, A, Yamada, M. Non-steroidal anti-inflammatory drugs have potent anti-fibrillogenic and fibril-destabilizing effects for alpha-synuclein fibrils in vitro. Neuropharmacology 2008;54:620-627.

Ho, YJ, Eichendorff, J, Schwarting, RK. Individual response profiles of male Wistar rats in animal models for anxiety and depression. Behav Brain Res 2002;136:1-12.

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;1043:179-185.

Imamura, K, Hishikawa, N, Ono, K, Suzuki, H, Sawada, M, Nagatsu, T, et al. Cytokine production of activated microglia and decrease in neurotrophic factors of neurons in the hippocampus of Lewy body disease brains. Acta Neuropathol 2005;109:141-150.

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;106:518-526.

Kadiu, I, Glanzer, JG, Kipnis, J, Gendelman, HE, Thomas, MP. Mononuclear

phagocytes in the pathogenesis of neurodegenerative diseases. Neurotox Res 2005;8:25-50.

Klegeris, A, McGeer, PL. Non-steroidal anti-inflammatory drugs (NSAIDs) and other anti-inflammatory agents in the treatment of neurodegenerative disease. Curr Alzheimer Res 2005;2:355-365.

Kurosaki, R, Muramatsu, Y, Kato, H, Araki, T. Biochemical, behavioral and immunohistochemical alterations in MPTP-treated mouse model of Parkinson's disease. Pharmacol Biochem Behav 2004;78:143-153.

Laatu, S, Revonsuo, A, Pihko, L, Portin, R, Rinne, JO. Visual object recognition deficits in early Parkinson's disease. Parkinsonism Relat Disord 2004;10:227-233.

Levin, BE, Llabre, MM, Reisman, S, Weiner, WJ, Sanchez-Ramos, J, Singer, C, et al. Visuospatial impairment in Parkinson's disease. Neurology 1991;41:365-369.

Levy, G, Tang, MX, Louis, ED, Cote, LJ, Alfaro, B, Mejia, H, et al. The association of incident dementia with mortality in PD. Neurology 2002;59:1708-1713.

Lewis, SJ, Slabosz, A, Robbins, TW, Barker, RA, Owen, AM. Dopaminergic basis for deficits in working memory but not attentional set-shifting in Parkinson's disease. Neuropsychologia 2005;43:823-832.

Li, JS, Chao, YS. Electrolytic lesions of dorsal CA3 impair episodic-like memory in rats. Neurobiol Learn Mem 2008;89:192-198.

McGeer, PL, Itagaki, S, Boyes, BE, McGeer, EG. Reactive microglia are positive for HLA-DR in the substantia nigra of Parkinson's and Alzheimer's disease brains. Neurology 1988;38:1285-1291.

McGeer, PL, McGeer, EG. Inflammation and neurodegeneration in Parkinson's disease. Parkinsonism Relat Disord 2004;10 Suppl 1:S3-7.

McGeer, PL, McGeer, EG. The inflammatory response system of brain: implications for therapy of Alzheimer and other neurodegenerative diseases. Brain Res Rev 1995;21:195-218.

Meissner, W, Prunier, C, Guilloteau, D, Chalon, S, Gross, CE, Bezard, E. Time-course of nigrostriatal degeneration in a progressive MPTP-lesioned macaque model of Parkinson's disease. Mol Neurobiol 2003;28:209-218.

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;211:13-16.

Mumby, DG, Tremblay, A, Lecluse, V, Lehmann, H. Hippocampal damage and anterograde object-recognition in rats after long retention intervals. Hippocampus 2005;15:1050-1056.

Ogura, K, Ogawa, M, Yoshida, M. Effects of ageing on microglia in the normal rat brain: immunohistochemical observations. Neuroreport 1994;5:1224-1226.

Owen, AM, Sahakian, BJ, Hodges, JR, Summers, BA, Polkey, CE, Robbins, TW. Dopamine-dependent frontostriatal planning deficits in early Parkinson's disease. Neuropsychology 1995;9:126-140.

Paxinos, G, Watson, C. The Rat Brain in Stereotaxic Coordinates. London: Academic Press, 1986.

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;195:322-329.

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;33:374-382.

Ramirez-Ruiz, B, Junque, C, Marti, MJ, Valldeoriola, F, Tolosa, E. Neuropsychological deficits in Parkinson's disease patients with visual hallucinations. Mov Disord 2006;21:1483-1487.

Reksidler, AB, Lima, MM, Dombrowski, P, Andersen, ML, Zanata, SM, Andreatini, R, et al. Repeated intranigral MPTP administration: a new protocol of prolonged locomotor impairment mimicking Parkinson's disease. J Neurosci Meth 2008;167:268-277.

Sanberg, PR, Bunsey, MD, Giordano, M, Norman, AB. The catalepsy test: its ups and downs. Behav Neurosci 1988;102:748-759.

Sawada, M, Imamura, K, Nagatsu, T. Role of cytokines in inflammatory process in Parkinson's disease. J Neural Transm Suppl 2006:373-381.

Schneider, JS, Tinker, JP, Van Velson, M, Giardiniere, M. Effects of the partial glycine agonist D-cycloserine on cognitive functioning in chronic low dose MPTP-treated monkeys. Brain Res 2000;860:190-194.

Sedelis, M, Schwarting, RK, Huston, JP. Behavioral phenotyping of the MPTP mouse model of Parkinson's disease. Behav Brain Res 2001;125:109-125.

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;20:670-682.

Stern, Y, Tetrud, JW, Martin, WR, Kutner, SJ, Langston, JW. Cognitive change following MPTP exposure. Neurology 1990;40:261-264.

West, WH, Tauer, KW, Yannelli, JR, Marshall, GD, Orr, DW, Thurman, GB, et al. Constant-infusion recombinant interleukin-2 in adoptive immunotherapy of advanced cancer. N Engl J Med 1987;316:898-905.

Wood, ER, Mumby, DG, Pinel, JP, Phillips, AG. Impaired object recognition memory in rats following ischemia-induced damage to the hippocampus. Behav Neurosci 1993;107:51-62.

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 Brain Res Protoc 2005;16:58-64.

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.

Figure Legends

Fig. 1. Effects of MPTP on catalepsy in the bar test. MPTP was bilaterally infused into the substantia nigra pars compacta (SNc), then the catalepsy bar test was performed on days 3, 5, 7, and 9 after the treatment. * $P < 0.05$, ** $P < 0.01$ compared to the sham-operated group on the same day. The data are expressed as the mean ± SEM for the indicated number of animals.

Fig. 2. Effects of MPTP on behavior in the object recognition test. (A) shows the percentage of time spent exploring object "B" or "D" during the test. (B) shows the rearing number during the test. Data are expressed as the mean ± SEM for the indicated number of animals. ** $P < 0.01$ compared to the sham-operated group at the same time point.

Fig. 3. Effects of MPTP on dopamine (DA)-containing neurons in the striatum and substantia nigra pars compacta (SNc). The brains were taken 28 days after MPTP treatment. DAergic neurons, stained by tyrosine hydroxylase (TH) immunoreaction, are shown in the representative coronal sections of the striatum (A, B) and SNc (C, D) (magnification, ×50; bar, 200 μm) in sham-operated and MPTP-treated rats. Black squares (24 585μm^2) in the schematic drawings are used for measuring optical density (OD) of TH immunoreactivity in the striatum and neuronal density (per mm^2) in the SNc. MPTP-treated rats show decreases in relative OD of TH immunoreactivity in the striatum (E) and in density of DAergic neurons in the SNc (F). * $P < 0.05$, ** $P < 0.01$, compared to

corresponding sham-operated rats. The schematic drawings are modified from the rat brain atlas (Paxinos and Watson, 1986).

Fig. 4. Effects of MPTP on microglial activation in the brain. The brains were taken 28 days after MPTP lesion. No activated microglia, OX-6-positive cells, was found in the substantia nigra pars compacta (SNc) and hippocampus (HIP) of sham-operated rats. MPTP treatment caused a massive accumulation of activated microglia in the SNc and HIP (magnification, ×50; bar, 200 μm). Insets show activated microglia at magnification of ×400, bar, 20μm.

Fig. 5. Effects of MPTP on neurons in the hippocampal CA1 area. The brains were taken 28 days after MPTP treatment. Images show Nissl-stained pyramidal neurons in the CA1 area of hippocampus, as indicated in the square of schematic drawing, in sham-operated (A) and MPTP-treated (B) rats (magnification, ×200; bar, 100 μm). MPTP-treated rats show decrease in neuronal area in the CA1 area, ** $p < 0.001$, compared to sham-operated rats (C).

Table 1.

Table 1. Effect of MPTP on behavior in the elevated plus-maze test on day 25.

	Sham (n=15)	MPTP (n=25)
Open arm latency (sec)	154.0 ± 36.9	242.0 ± 20.2*
Open arm time (sec)	37.4 ± 12.5	11.0 ± 4.6*
Enclosed arm time (sec)	191.7 ± 21.0	248.6 ± 10.2*
Open arm entries (no.)	4.1 ± 1.1	1.4 ± 0.5*
Enclosed arm entries (no.)	8.1 ± 1.4	5.9 ± 0.8
Total distance (cm)	2 270.8 ± 265.7	1 934.9 ± 192.7
Total rearing (no.)	16.9 ± 1.3	14.7 ± 1.0

* $p < 0.05$, compared to the sham-operated group. The data are expressed as the mean ± SEM for the indicated number of animals.

Table 2.

Table 2. Correlations between behavior in the elevated plus-maze and object recognition tests.

	Object recognition					
	Total exploratory time (sec)		% D exploration time		Rearing (no.)	
Elevated plus-maze	Pearson Correlation	*p* value	Pearson Correlation	*p* value	Pearson Correlation	*p* value
Open arm latency (sec)	-0.172	0.289	-0.03	0.856	-0.152	0.348
Open arm time (sec)	-0.072	0.661	0.321	0.043*	0.167	0.302
Enclosed arm time (sec)	-0.126	0.44	-0.39	0.013*	-0.33	0.037*
Open arm entries (no.)	-0.008	0.96	0.314	0.049*	0.104	0.523
Enclosed arm entries (no.)	0.284	0.076	0.177	0.275	-0.033	0.841
Total distance (cm)	0.171	0.403	0.108	0.601	-0.091	0.658
Total rearing (no.)	0.355	0.025	0.034	0.836	0.289	0.07

P values, 2-tailed.

Fig. 1.

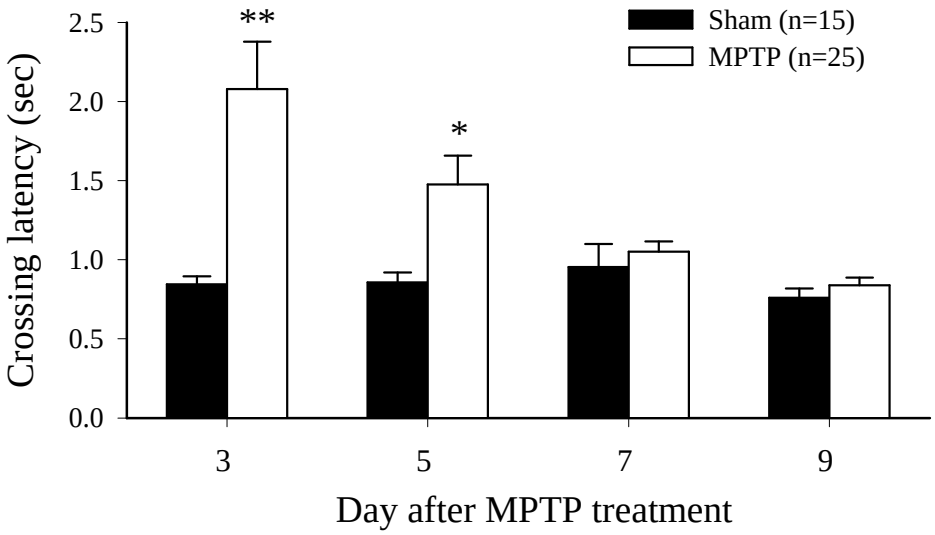

Crossing latency (sec)
2.5
2.0
1.5
1.0
0.5
0.0
**
*
Sham (n=15)
MPTP (n=25)
3
5
7
9
Day after MPTP treatment

Fig. 2.

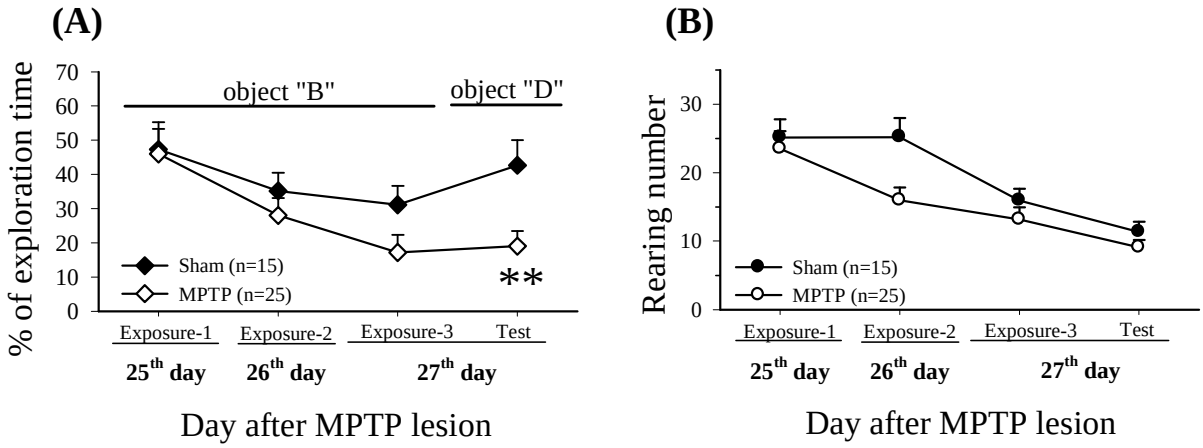

(A)
% of exploration time
70
60
50
40
30
20
10
0
object "B"
object "D"
Sham (n=15)
MPTP (n=25)
**
Exposure-1
Exposure-2
Exposure-3
Test
25th day
26th day
27th day
Day after MPTP lesion
(B)
Rearing number
30
20
10
0
Sham (n=15)
MPTP (n=25)
Exposure-1
Exposure-2
Exposure-3
Test
25th day
26th day
27th day
Day after MPTP lesion

Fig. 3.

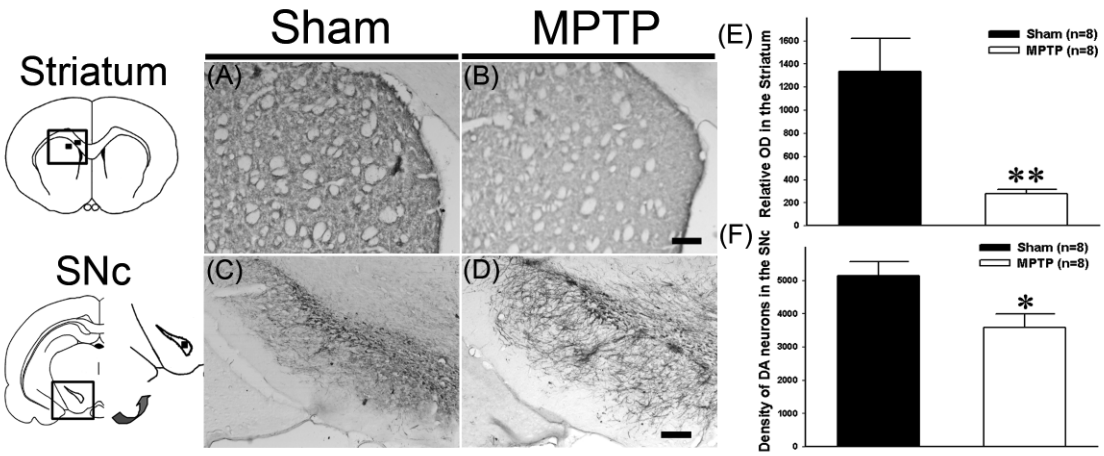

Sham
MPTP
Striatum
SNc
(A)
(B)
(C)
(D)
(E)
(F)
Sham (n=8)
MPTP (n=8)
Relative OD in the Striatum
Density of DA neurons in the SNc
**
*

Fig. 4.

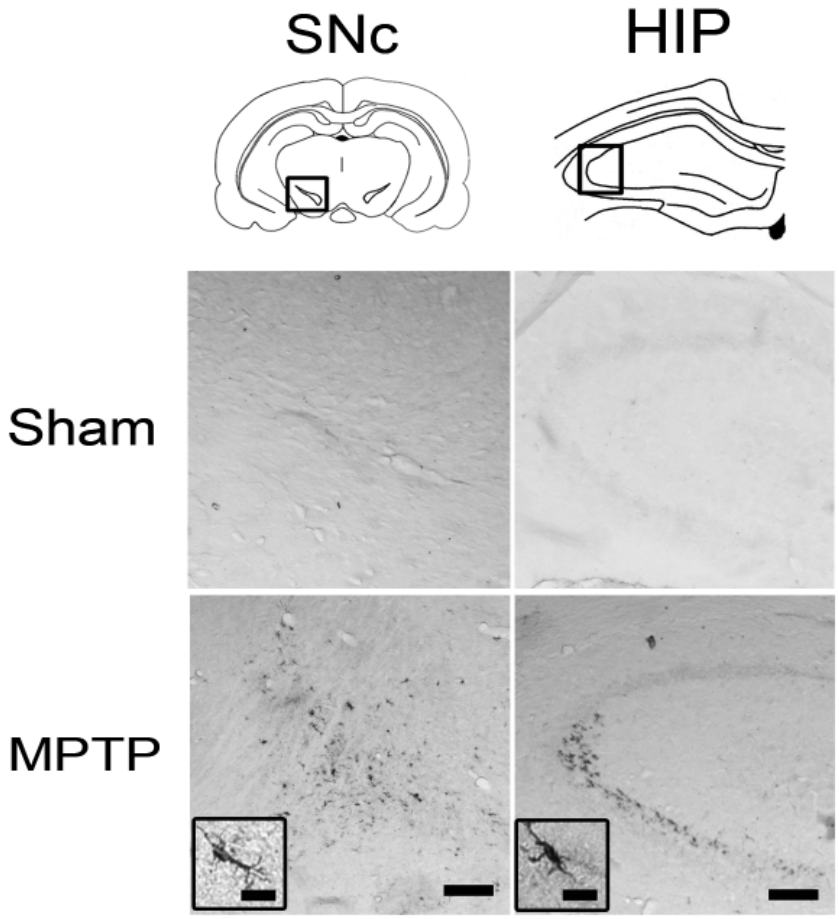
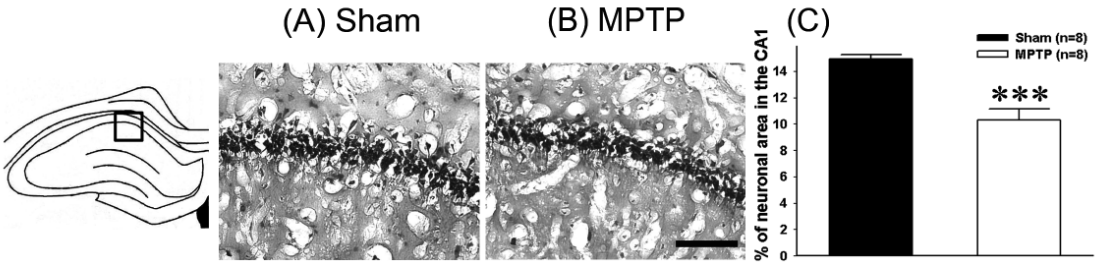

SNc
HIP
Sham
MPTP

Fig. 5.


(A) Sham
(B) MPTP
(C)
Sham (n=8)
MPTP (n=8)
***
% of neuronal area in the CA1
0
2
4
6
8
10
12
14

MPTP-induced dopaminergic degeneration and deficits in object recognition are accompanied with neuroinflammation in the hippocampus

Hiu-Ngar Sy[1#], Shey-Lin Wu[1, 2#], Wen-Fu Wang[1], An-Li Wang[3], Yao-Ting Huang[1], Ying-Ming Liou[3], Cornelius Rainer Pawlak[4]*, Ying-Jui Ho[5]*

[1] Department of Neurology, Chang-Hua Christian Hospital, Taiwan, ROC

[2] Department of Bioindustry Technology, Dayeh University, Taiwan, ROC

[3] Department of Life Sciences, National Chung Hsing University, Taiwan, ROC

[4] Institute of Neuropsychology and Clinical Psychology, Central Institute of Mental Health, Mannheim, Germany

[5] School of Psychology, Chung Shan Medical University, Taiwan, ROC

[#]equal contribution

***Corresponding authors:**

Ying-Jui Ho Ph.D.

School of Psychology, Chung Shan Medical University, Taiwan, ROC

Address: No. 110, Sec. 1, Jianguo N. Rd., Taichung City 402, Taiwan, ROC

E-mail: yjho@csmu.edu.tw; joshuayjho@yahoo.com.tw

Tel: +886-4-24730022 ext. 11858; Fax: +886-4-23248191

Cornelius Rainer Pawlak Ph.D.

Institute of Neuropsychology and Clinical Psychology, Central Institute of Mental Health, Mannheim, Germany

Address: J5, D-68159 Mannheim, Germany

E-mail: cornelius.pawlak@zi-mannheim.de

Tel: +49 (0) 621 1703 6262; Fax: +49 (0) 621 1703 6255

Abstract

Emotional changes, impairment on object recognition, and neuroinflammation are seen in Parkinson's disease with dementia (PDD). Here, we showed that bilateral infusion of 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) into the rat substantia nigra pars compacta (SNc) of Wistar rats caused degeneration of nigrostriatal dopaminergic neurons, microglial activation in the SNc and hippocampus, as well as cell loss in the hippocampal CA1 area. With regard to behavior, increase of anxiety-like behavior and impairment of object recognition were observed during the fourth week after the MPTP lesion. The behavioral changes were not caused by motor impairment, since the rats had already recovered from MPTP-induced catalepsy before the tests were performed. These findings show that MPTP-induced neuroinflammation and its consequences, for example, microglial activation and cell loss in the hippocampus, may contribute to dopaminergic degeneration-related behavioral deficits and suggest that, in addition to the dopaminergic system, the limbic system may also participate in the pathophysiology of PDD.

Keywords: Parkinson's disease, dementia, object recognition, neuroinflammation, microglial activation, MPTP

1. Introduction

Parkinson's disease (PD) is a high prevalent neurodegenerative disorder (Aarsland et al., 2005). In addition to the motor dysfunctions, cognitive impairment and dementia are seen in a high percentage of PD patients (Brown and Marsden, 1984; Owen et al., 1995). The proportion of PD patients with dementia is 25-30%, an incidence up to six times higher than that in healthy people (Aarsland et al., 2001). Emotional changes (Levin et al., 1991), for example elevation of anxiety levels (Fenelon et al., 2000), visuospatial dysfunctions (Crucian and Okun, 2003; Emre, 2003), as well as impairments on facial recognition and object perception (Barnes et al., 2003; Laatu et al., 2004; Ramirez-Ruiz et al., 2006) are the main symptoms in Parkinson's disease dementia (PDD).

Although the loss of dopamine (DA)-containing neurons originated in the substantia nigra is the main character of PD (McGeer and McGeer, 2004), inflammation has also been proposed as a possible mechanism in the pathogenesis of PD, as the intensity of inflammation in the substantia nigra is considerably higher in PD patients than in controls (Abramsky and Litvin, 1978; McGeer and McGeer, 2004). Nigrostriatal dysfunction alone is probably not sufficient for the development of dementia in PD because activated microglia have been observed not only in the substantia nigra and putamen, where DA loss is prominent, but also in the hippocampus of PD patients (McGeer et al., 1988; McGeer and McGeer, 1995; Sawada et al., 2006). Microglial activation in the hippocampus has been suggested to be responsible for functional changes in neurons and cognitive decline in PD and dementia with Lewy bodies (Imamura et al., 2005). Emotional changes and cognitive disturbances, particularly in tests involving spatial learning and memory, are linked with aberrations in the

mesocorticolimbic and striatal systems (Caraceni et al., 1993; Denicoff et al., 1987; West et al., 1987). Thus, microglial activation in these areas may also be involved in the pathophysiology of PDD.

Literatures have reported deficits in habit learning and spatial memory in the active avoidance test (Da Cunha et al., 2001) and in water maze test (Da Cunha et al., 2002; Da Cunha et al., 2003), respectively, in MPTP-induced rat PD model. Disturbance in visual discrimination in MPTP-treated monkeys has also been indicated (Schneider et al., 2000). However, no data are available in object recognition in MPTP-treated rodents, which would better model the disability of object recognition in PDD. To better understand the pathophysiology of PDD, it is necessary to detect neuronal changes occur after degeneration of the nigrostriatal DAergic system. Animals treated with 1-methyl-4-phenyl-1,2,3,6-terahydropyriding (MPTP) are widely used as a PD animal model, as MPTP selectively destroys the DAergic system and causes microglial activation in humans (Mogi et al., 1996). Impairment of executive and visuospatial functions is observed not only in patients with PDD (Crucian and Okun, 2003; Emre, 2003) but also in people exposed to MPTP (Stern et al., 1990). However, it is not known whether the MPTP-induced inflammation and behavioral changes seen in animal studies resemble the pathophysiology and symptoms seen in PDD. We examined motor and emotional behavior as well as object recognition in rats after MPTP lesion using a battery of behavioral tests, namely a bar test as a measure of catalepsy (Sanberg et al., 1988), an elevated plus-maze as a measure of anxiety-like behavior (Ho et al., 2002), and an object recognition test in an open field (Mumby et al., 2005). In addition, we analyzed microglial activation and cell loss in the brain. The results showed that MPTP-induced DAergic degeneration and deficits in emotional behavior and object

recognition were accompanied with neuroinflammation in the hippocampus. It is thus suggested that, in addition to the DAergic system, the limbic system may also participate in the pathophysiology of PDD.

2. Methods

2.1 Animals

Male Wistar rats (384 ± 4.5 g; n = 40; National Laboratory Animal Center, ROC) were housed in groups of five in acrylic cages (35 × 56 × 19 cm) in an animal room with a 12 h light-dark cycle (lights on at 07:00 h) 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 the experimental procedures were performed according to the NIH Guide for Care and Use of Laboratory Animals and were approved by the Animal Care Committee of Chung Shan Medical University (IACUC approval NO: 699).

2.2 General procedures

All animals underwent stereotaxic surgery and bilateral infusion into the substantia nigra pars compacta (SNc) of either MPTP-HCl (1 μmol in 2 μl of saline; Sigma, USA) or vehicle (day 0) (see surgery section below). They were then subjected to a bar test on days 3, 5, 7, and 9 and an elevated plus-maze test on day 25, followed by an object recognition test starting on day 25 and finishing on day 27, all of which were started at least 2 h after the beginning of the light-phase (7:00 h). Behavioral tests were performed in a dim observation room (28 lux red light). The test equipment and object were cleaned using 20% ethanol and thoroughly dried before each test trial. On day 28 after MPTP treatment, the rats were sacrificed by exposure to CO_2, transcardially perfused with phosphate-buffered saline (PBS) and followed by 4% paraformaldehyde

in PBS. The brains were immediately removed and post-fixed in 20% sucrose solution with 4% paraformaldehyde at 4°C for histochemical assessment.

2.3. Surgery

Brain surgery was performed on a stereotaxic instrument. The rats received intraperitoneal injection (IP) of atropine sulfate (0.4 mg/kg) to suppress salivation and were anesthetized using Zoletil (2 mg/kg, IP; Virbac, Carros, France). In the lesion group (n = 25), MPTP-HCl (1 μmol in 2 μl of saline) was bilaterally infused into the SNc through a 30 gauge stainless needle at a rate of 0.7 μl/min at a site with the following coordinates adapted from the rat brain atlas (Paxinos and Watson, 1986): AP -5.0 mm, ML ± 1.8 mm, DV -8.0 mm from the bregma. Sham-operated rats were subjected to the same procedure, but were infused with 2 μl of saline instead of MPTP (n = 15). Immediately after surgery, the rats received a muscular injection with penicillin-G procaine (0.2 ml, 20,000 IU) and housed individually in plastic cages (25 × 41 × 19 cm) for 10 days, then 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 (Da Cunha et al., 2001; Ferro et al., 2005).

2.4. Behavioral tests

Behavior in the bar test was scored manually by a trained observer blind to the treatment conditions. The elevated plus-maze and object recognition test was monitored and scored by using a video camera positioned above the apparatus and a home-made video image analysis system (VIAS) (Li and Chao, 2008). The spatial resolution of the VIAS was set to 0.7 cm; and the image processing capability was higher than 14 pictures/sec.

Bar test: The bar test was performed on days 3, 5, 7, and 9 after MPTP lesion. Catalepsy was measured as the mean time spent by a rat to climb over a 9 cm high bar after being laid across it with its hind limbs on the floor (Sanberg et al., 1988). Each animal was tested in 3 consecutive trials on each trial day.

Elevated plus-maze test: Unconditioned anxiety-like avoidance behavior was assessed as our previous report, using the elevated plus-maze test (Ho et al., 2005), performed on day 25. The measures recorded were: (1) open arm latency, that is, the time from placing the rat into the plus-maze until it entered one of the open arms, (2) the time spent on and (3) the number of entries into open or enclosed arms, (4) total distance, that is, the distance traveled by the rat in cm, and (5) rearing number.

Object recognition test: The apparatus and testing procedure for the object recognition test were similar to those described previously (Mumby et al., 2005). An open field arena was constructed of black polyvinyl plastic (100 cm long × 100 cm wide × 60 cm high). Each rat was subjected to 3 exposure sessions at 24 h intervals. Five minutes after the last exposure session, a test trial was performed. Four different objects made of transparent glass, paper, porcelain, or metal (all sizes around 10 × 10 × 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. On day 25 (5 min after the elevated plus-maze test), the rat was placed in the only free corner and allowed to explore the objects for a 5 min exposure session, and also on the next 2 consecutive days. Five minutes after the last exposure session, object “B” was replaced by a novel object “D” and the animal was returned to the open field for a 5 min test session. The time spent exploring the objects and the number of rearing during the exposure and test sessions were recorded. The percentage

of the exploration time spent on object B or D in the sessions [(Time $_{B \text{ or } D}$ / Time $_{all\ objects}$) × 100%] was calculated. The percentage of time spent exploring the novel object "D" served as the measure of recognition memory for the familiar object. Exploration of an object was defined as the rat approaching an object and having physical contact with it, either with its snout and/or forepaws.

2.5. Histological assay

To detect DAergic degeneration and microglial activation, frozen coronal brain sections (30 μm) were cut, rinsed in PBS, picked up on gelatinized slides, and immunostained at 4°C overnight with mouse monoclonal antibodies against rat tyrosine hydroxylase (TH) (1:4 000; Zymade, USA) or rat MHC class II (OX-6; 1:400; BD Biosciences Pharmingen, CA, USA) diluted in PBS. OX-6 selectively stains activated microglia (Ogura et al., 1994). The sections were incubated sequentially for 30 min at 37ºC with biotinylated horse anti-mouse IgG antibody (Vector Laboratory, CA, USA) and avidin-biotin-horseradish peroxidase complex (ABC Elite HRP kit; Vector Laboratory, CA, USA), then were incubated for 30 min at room temperature with 0.02% 3,3'-diaminobenzidine (Sigma, USA). The reaction was stopped by extensive washing with PBS. To detect cell loss, Nissl staining was used to identify neurons.

Image analysis: The stained brain sections, identified according to the rat brain atlas (Paxinos and Watson, 1986), were used for measuring histological changes with the methods described previously (Xavier et al., 2005), using a microscope (ZEISS AXioskop2, Germany) coupled to a CCD (Optronics, USA) and the Image Pro Plus Software 4.1 (Media Cybernetics, CA, USA). In this study, an area of interest were created, measuring 24 585 μm^2, to determine the optical density of TH immunoreactivity in the striatum and neuronal density in the SNc, and 40 000 μm^2 for

counting the neuronal density in the hippocampal CA1 area. To measure the intensity of DAergic projections in the striatum, the images of TH staining were converted to gray scale. The gray level of 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. To measure the density of DAergic neurons in the SNc, the images were captured, 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. Because the neurons are tightly packed, it was difficult to directly calculate the number of pyramidal neurons in the CA1 area from a 30 μm-thick brain section. Thus, a semi-quantitative method, percentage of area of Nissl-stained neurons in an area of interest in the CA1 area, was used to represent the neuronal density.

2.6. Data analysis

Statistical testing was performed to compare the two groups using the *t*-test. Analysis of variance (ANOVA) for repeated measures, followed by Scheffé's post hoc test, and evaluation of behavioral correlations were used when appropriate. All results are expressed as the mean ± SEM. The level of significance was defined as $P < 0.05$ (two-tailed).

3. Results

Behavior: After MPTP lesion, the animals showed a significant and transitory catalepsy, i.e., an increase in the crossing latency in the bar test. ANOVA with repeated measures revealed significant main effects of time ($F(3,114) = 7.03$, $P < 0.001$) and lesion ($F(1,38) = 10.05$, $P < 0.01$) as well as time×lesion interaction ($F(3,114) = 6.66$, P

< 0.001). Significant longer crossing latencies in the MPTP-treated animals were seen on days 3 and 5 after MPTP lesion (d.f. = 38, *t*–values ≧ 2.54, *P*-values < 0.05), while, on days 7 and 9, MPTP- and sham-treated rats displayed comparable crossing latencies (Fig. 1.).

【Figure 1 should be here】

An increased open arm latency, decreased open arm time, and less number of open arm entries in the elevated plus-maze test, compared to the controls, were seen in MPTP-treated rats on day 25 (d.f. = 38, *t*-values > 1.98, *P*-values < 0.05), indicating higher anxiety-like levels. However, the number of rearing, enclosed arm entries, and total distance traveled by the rats in the elevated plus-maze test, which are commonly used to measure general activity, were not different between the groups (Table 1).

【Table 1 should be here】

MPTP-treated rats spent a lower percentage of time exploring the novel object "D" on day 27 after MPTP lesion than the sham-operated controls (d.f. = 38, $t = 2.94$, $P < 0.01$) (Fig. 2A). As shown in Fig. 2B, ANOVA with repeated measures revealed a significant time effect ($F(1,114) = 24.86$, $P < 0.001$) for rearing number, but no significant lesion effect or time×lesion interaction.

【Figure 2 should be here】

The open arm time and number of open arm entries in the elevated plus-maze test were significantly correlated with percentage of time spent on exploring novel object (*P*-values < 0.05). However, enclosed arm time in the elevated plus-maze test was negatively correlated with percentage of time spent on exploring novel object and the number of rearing in the object recognition test (*P*-values < 0.05) (Tab. 2.).

【Table 2 should be here】

Histology: Representative photomicrographs of immunostained and Nissl-stained brain sections are shown in Figs. 3-5. TH positive neurons were found in the striatum and SNc of the brain from sham-operated and MPTP-treated groups. TH immunoreactivity was observed in neuronal cell bodies and their processes. MPTP treatment induced a reduction in relative optical density of TH immunoreactivity in the striatum (df = 14, t = 3.71, $P < 0.01$) and a decrease in density of DAergic neurons in the SNc (df = 14, t = 2.62, $P < 0.05$), compared to the sham-operated group (Fig. 3). Microglial activation, indicated by an accumulation of OX-6-positive cells, was evident in the SNc and hippocampus (Fig. 4), but not in the prefrontal cortex, striatum, or cerebral cortex (data not shown). Semi-quantitative analysis confirmed that MPTP lesion decreased the neuronal density in the pyramidal cell layer in the hippocampal CA1 area (df = 14, t = 4.92, $P < 0.001$), compared to the sham-operated rats (Fig. 5).

【Figure 3-5 should be here】

4. Discussion

Four weeks after intra-SNc infusion of MPTP, there were neurodegeneration of the nigrostriatal DAergic system, cell loss in the hippocampal CA1 area, as well as microglial activation in the SNc and hippocampus. Furthermore, emotional-related and cognitive changes were also observed during the fourth week after MPTP lesion, namely, increased anxiety-like avoidance behavior and impairment of object recognition. We did not find evidence of motor impairment 7 days after MPTP lesion when catalepsy was no longer observed, suggesting that the behavioral differences seen in subsequent tests were not due to motor deterioration. These results suggest that the MPTP-induced neuroinflammation and cell loss in the hippocampus may contribute to

DAergic degeneration-related behavioral deficits.

MPTP is a toxin commonly used to induce PD animal model because it causes specific degeneration of DAergic neurons in the substantia nigra and the loss of nerve terminals in the striatum. In the current study, intra-SNc infusion of MPTP resulted in a significant decrease in TH immunohistochemical staining in the SNc and striatum, indicating DAergic lesion. Although calculating the cell number in the representative brain sections yielded similar histological results reported in the literature, stereologic approach by counting cells in a complete series of sections can provide additional data (Ferro et al., 2005; Meissner et al., 2003). MPTP-lesioned rats have been reported showing lower than controls in spontaneous locomotion in the open field test one day after the surgery (Capitelli et al., 2008; Perry et al., 2005; Reksidler et al., 2008). However, the behavioral changes were no longer observed 7 (Capitelli et al., 2008) and 18 days (Ferro et al., 2005; Perry et al., 2005) after the lesion. Further, in line with previous reports showing transitory catalepsy by using the bar test (Ferro et al., 2005; Sedelis et al., 2001), catalepsy was seen during the first 5 days, but not on days 7 and 9 after MPTP lesion. Recovery of motor function was further supported by the lack of differences between the groups in the rearing number, enclosed arm entries, and distance traveled by the rats in the elevated plus-maze test, as well as rearing number in the object recognition test, indicating absence of gross motor impairment and suggesting that the behavioral performance in the tests was not confounded by motor impairment.

In consistent with the fact that high anxiety levels have been indicated in the late course of PD (Fenelon et al., 2000), MPTP lesion caused an enhancement of anxiety-like behavior in the elevated plus-maze test, indicated as lowered open arm time

and increased enclosed arm time. In addition, dysfunction of object recognition in animal model may be compatible with some phenomenon of visuospatial (Girotti et al., 1988), facial recognition, and object perception deficits (Barnes et al., 2003; Ramirez-Ruiz et al., 2006) in PD patients. Interestingly, anxiety levels were negatively correlated with the time spent exploring novel object but not correlated with total exploration time. This result was consistent with the findings that cognitive despaired PD patients show co-existence of anxiety disorders (Fenelon et al., 2000). It has been reported that cognitively deteriorated PD patients performed more poorly than the healthy controls and cognitively preserved PD patients in discriminating objects, where the subjects used visual sensation for the object recognition task because they need to response to the stimuli, pictures or written words, presented on a screen (Laatu et al., 2004). It is noteworthy that the animals in the current study may not totally rely on visual sensation to recognize the objects. Further, it has been demonstrated that MPTP-treated rats show deficits in performing a spatial working memory in the Morris water maze task (Ferro et al., 2005), and in acquisition and retention processes in an active avoidance test (Da Cunha et al., 2001), suggesting that MPTP-treated rats may be a model for early Parkinson's disease amnesia. The hippocampus is important for spatial navigation (Zhang et al., 2004), recognition memory (Broadbent et al., 2004), and short-term memory associating objects and their locations (Li and Chao, 2008; Piekema et al., 2006). Moreover, the hippocampal CA1 region plays a critical role in object recognition because ischemia-induced cell loss in the hippocampal CA1 area of rats produced object recognition deficits in the acquisition and retention in delayed nonmatching-to-sample task (Wood et al., 1993). Furthermore, local pharmacological manipulations in the CA1 area are also affecting consolidation of object recognition

memory (Clarke et al., 2008; de Lima et al., 2006). In agree with the above findings, our present data showing that MPTP lesion causes inflammation and cell loss in the hippocampal CA1 pyramidal neurons may provide possible neuronal mechanisms underling the MPTP-induced object recognition impairment and may prove to be a useful tool in assessing the ability of pharmacological agents to prevent neurodegeneration-related recognition deficits.

Immunohistochemical alterations in the brain have been suggested to be involved the behavioral changes seen after DAergic degeneration (Kurosaki et al., 2004). Although DAergic deficit is the main neurochemical impairment in PD, clinical data show that dementia in PD patients does not improve with levodopa treatment (Lewis et al., 2005) and that motor symptoms and cognitive dysfunction in PDD patients are correlated strongly with non-DAergic systems (Levy et al., 2002). Microglia, the resident immune cells of the central nervous system, act as regulators of the secretion of neurotrophic and neurotoxic factors (Kadiu et al., 2005). Sawada et al. have proposed that activated microglia may change *in vivo* from neuroprotective to neurotoxic subsets as degeneration of DAergic neurons in the substantia nigra progresses in PD (Sawada et al., 2006). Chronic activation of microglia causes inflammatory responses and may, through the release of cytokines, lead to neuronal damage (Dheen et al., 2007; Imamura et al., 2003). Microglial activation and increased levels of inflammatory cytokines have been observed in the substantia nigra, putamen, and hippocampus in PD patients (McGeer et al., 1988; McGeer and McGeer, 1995; Sawada et al., 2006). Furthermore, neuroinflammation has been suggested to be responsible for neuronal degeneration and cognitive decline in PD and dementia with Lewy bodies (Imamura et al., 2005). Similarly, in the present study, MPTP caused a chronic activation of microglia in the

SNc and hippocampus, which may participate in the MPTP-induced emotional and cognitive deficits, notwithstanding the fact that other factors are probably also involved. The mechanisms by which degeneration of DAergic neurons causes neuroinflammation in the limbic system need to be studied further. In addition, non-steroidal anti-inflammatory drugs are able to reduce neuronal death induced by activated microglia and improve the motor impairment in PD (Hirohata et al., 2008; Klegeris and McGeer, 2005). Thus, suppression of microglial activation by using anti-inflammatory compounds may therefore be a feasible strategy for preventing neuronal and cognitive decline in PD (Sriram et al., 2006).

In addition to the DAergic degeneration in the SNc and striatum, consistent with the pathophysiology of PD, intra-nigral infusion of MPTP also caused increases in microglial activation in the brain, cell loss in the hippocampal CA1 area, emotional changes, and object recognition deficits. These findings show that MPTP-induced neuroinflammation and its consequences may contribute to DAergic degeneration-related behavioral deficits and suggest that, in addition to the DAergic system, the limbic system may also participate in the pathophysiology of PDD.

Acknowledgments

The authors thank Drs. Yuan-Feen Tsai and Rainer K. Schwarting for their thoughtful comments on this manuscript, Dr. Jay-Shake Li and Mr. Sheng-Yuan Wang for kindly providing their self-developed software to collect the behavioral data, and also Ms. Shih-Chun Ho for her technical assistance. This work was supported by grants from the National Science Council of the ROC (NSC 96-2320-B-040-019; DAAD/NSC

Project Based Personnel Exchange Program 0950042882P), Chang-Hua Christian Hospital (CCH grant 93118 and 92137), Chung Shan Medical University (097-CCH-CSMU-14), and German Research Foundation (DFG PA 818/4-1).

References

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;56:730-736.

Aarsland, D, Zaccai, J, Brayne, C. A systematic review of prevalence studies of dementia in Parkinson's disease. Mov Disord 2005;20:1255-1263.

Abramsky, O, Litvin, Y. Automimmune response to dopamine-receptor as a possible mechanism in the pathogenesis of Parkinson's disease and schizophrenia. Perspect Biol Med 1978;22:104-114.

Barnes, J, Boubert, L, Harris, J, Lee, A, David, AS. Reality monitoring and visual hallucinations in Parkinson's disease. Neuropsychologia 2003;41:565-574.

Broadbent, NJ, Squire, LR, Clark, RE. Spatial memory, recognition memory, and the hippocampus. Proc Natl Acad Sci U S A 2004;101:14515-14520.

Brown, RG, Marsden, CD. How common is dementia in Parkinson's disease? Lancet 1984;2:1262-1265.

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;594:101-108.

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:1266-1269.

Clarke, JR, Rossato, JI, Monteiro, S, Bevilaqua, LR, Izquierdo, I, Cammarota, M. Posttraining activation of CB1 cannabinoid receptors in the CA1 region of the

dorsal hippocampus impairs object recognition long-term memory. Neurobiol Learn Mem 2008;90:374-381.

Crucian, GP, Okun, MS. Visual-spatial ability in Parkinson's disease. Front Biosci 2003;8:s992-997.

Da Cunha, C, Angelucci, ME, Canteras, NS, Wonnacott, S, Takahashi, RN. The lesion of the rat substantia nigra pars compacta dopaminergic neurons as a model for Parkinson's disease memory disabilities. Cell Mol Neurobiol 2002;22:227-237.

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.

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;79:236-242.

de Lima, MN, Luft, T, Roesler, R, Schroder, N. Temporary inactivation reveals an essential role of the dorsal hippocampus in consolidation of object recognition memory. Neurosci Lett 2006;405:142-146.

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.

Dheen, ST, Kaur, C, Ling, EA. Microglial activation and its implications in the brain diseases. Curr Med Chem 2007;14:1189-1197.

Emre, M. Dementia associated with Parkinson's disease. Lancet Neurol 2003;2:229-237.

Fenelon, G, Mahieux, F, Huon, R, Ziegler, M. Hallucinations in Parkinson's disease:

prevalence, phenomenology and risk factors. Brain 2000;123 (Pt 4):733-745.

Ferro, MM, Bellissimo, MI, Anselmo-Franci, JA, Angellucci, 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.

Girotti, F, Soliveri, P, Carella, F, Piccolo, I, Caffarra, P, Musicco, M, et al. Dementia and cognitive impairment in Parkinson's disease. J Neurol Neurosur Ps 1988;51:1498-1502.

Hirohata, M, Ono, K, Morinaga, A, Yamada, M. Non-steroidal anti-inflammatory drugs have potent anti-fibrillogenic and fibril-destabilizing effects for alpha-synuclein fibrils in vitro. Neuropharmacology 2008;54:620-627.

Ho, YJ, Eichendorff, J, Schwarting, RK. Individual response profiles of male Wistar rats in animal models for anxiety and depression. Behav Brain Res 2002;136:1-12.

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;1043:179-185.

Imamura, K, Hishikawa, N, Ono, K, Suzuki, H, Sawada, M, Nagatsu, T, et al. Cytokine production of activated microglia and decrease in neurotrophic factors of neurons in the hippocampus of Lewy body disease brains. Acta Neuropathol 2005;109:141-150.

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;106:518-526.

Kadiu, I, Glanzer, JG, Kipnis, J, Gendelman, HE, Thomas, MP. Mononuclear

phagocytes in the pathogenesis of neurodegenerative diseases. Neurotox Res 2005;8:25-50.

Klegeris, A, McGeer, PL. Non-steroidal anti-inflammatory drugs (NSAIDs) and other anti-inflammatory agents in the treatment of neurodegenerative disease. Curr Alzheimer Res 2005;2:355-365.

Kurosaki, R, Muramatsu, Y, Kato, H, Araki, T. Biochemical, behavioral and immunohistochemical alterations in MPTP-treated mouse model of Parkinson's disease. Pharmacol Biochem Behav 2004;78:143-153.

Laatu, S, Revonsuo, A, Pihko, L, Portin, R, Rinne, JO. Visual object recognition deficits in early Parkinson's disease. Parkinsonism Relat Disord 2004;10:227-233.

Levin, BE, Llabre, MM, Reisman, S, Weiner, WJ, Sanchez-Ramos, J, Singer, C, et al. Visuospatial impairment in Parkinson's disease. Neurology 1991;41:365-369.

Levy, G, Tang, MX, Louis, ED, Cote, LJ, Alfaro, B, Mejia, H, et al. The association of incident dementia with mortality in PD. Neurology 2002;59:1708-1713.

Lewis, SJ, Slabosz, A, Robbins, TW, Barker, RA, Owen, AM. Dopaminergic basis for deficits in working memory but not attentional set-shifting in Parkinson's disease. Neuropsychologia 2005;43:823-832.

Li, JS, Chao, YS. Electrolytic lesions of dorsal CA3 impair episodic-like memory in rats. Neurobiol Learn Mem 2008;89:192-198.

McGeer, PL, Itagaki, S, Boyes, BE, McGeer, EG. Reactive microglia are positive for HLA-DR in the substantia nigra of Parkinson's and Alzheimer's disease brains. Neurology 1988;38:1285-1291.

McGeer, PL, McGeer, EG. Inflammation and neurodegeneration in Parkinson's disease. Parkinsonism Relat Disord 2004;10 Suppl 1:S3-7.

McGeer, PL, McGeer, EG. The inflammatory response system of brain: implications for therapy of Alzheimer and other neurodegenerative diseases. Brain Res Rev 1995;21:195-218.

Meissner, W, Prunier, C, Guilloteau, D, Chalon, S, Gross, CE, Bezard, E. Time-course of nigrostriatal degeneration in a progressive MPTP-lesioned macaque model of Parkinson's disease. Mol Neurobiol 2003;28:209-218.

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;211:13-16.

Mumby, DG, Tremblay, A, Lecluse, V, Lehmann, H. Hippocampal damage and anterograde object-recognition in rats after long retention intervals. Hippocampus 2005;15:1050-1056.

Ogura, K, Ogawa, M, Yoshida, M. Effects of ageing on microglia in the normal rat brain: immunohistochemical observations. Neuroreport 1994;5:1224-1226.

Owen, AM, Sahakian, BJ, Hodges, JR, Summers, BA, Polkey, CE, Robbins, TW. Dopamine-dependent frontostriatal planning deficits in early Parkinson's disease. Neuropsychology 1995;9:126-140.

Paxinos, G, Watson, C. The Rat Brain in Stereotaxic Coordinates. London: Academic Press, 1986.

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;195:322-329.

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;33:374-382.

Ramirez-Ruiz, B, Junque, C, Marti, MJ, Valldeoriola, F, Tolosa, E. Neuropsychological deficits in Parkinson's disease patients with visual hallucinations. Mov Disord 2006;21:1483-1487.

Reksidler, AB, Lima, MM, Dombrowski, P, Andersen, ML, Zanata, SM, Andreatini, R, et al. Repeated intranigral MPTP administration: a new protocol of prolonged locomotor impairment mimicking Parkinson's disease. J Neurosci Meth 2008;167:268-277.

Sanberg, PR, Bunsey, MD, Giordano, M, Norman, AB. The catalepsy test: its ups and downs. Behav Neurosci 1988;102:748-759.

Sawada, M, Imamura, K, Nagatsu, T. Role of cytokines in inflammatory process in Parkinson's disease. J Neural Transm Suppl 2006:373-381.

Schneider, JS, Tinker, JP, Van Velson, M, Giardiniere, M. Effects of the partial glycine agonist D-cycloserine on cognitive functioning in chronic low dose MPTP-treated monkeys. Brain Res 2000;860:190-194.

Sedelis, M, Schwarting, RK, Huston, JP. Behavioral phenotyping of the MPTP mouse model of Parkinson's disease. Behav Brain Res 2001;125:109-125.

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;20:670-682.

Stern, Y, Tetrud, JW, Martin, WR, Kutner, SJ, Langston, JW. Cognitive change following MPTP exposure. Neurology 1990;40:261-264.

West, WH, Tauer, KW, Yannelli, JR, Marshall, GD, Orr, DW, Thurman, GB, et al. Constant-infusion recombinant interleukin-2 in adoptive immunotherapy of advanced cancer. N Engl J Med 1987;316:898-905.

Wood, ER, Mumby, DG, Pinel, JP, Phillips, AG. Impaired object recognition memory in rats following ischemia-induced damage to the hippocampus. Behav Neurosci 1993;107:51-62.

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 Brain Res Protoc 2005;16:58-64.

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.

Figure Legends

Fig. 1. Effects of MPTP on catalepsy in the bar test. MPTP was bilaterally infused into the substantia nigra pars compacta (SNc), then the catalepsy bar test was performed on days 3, 5, 7, and 9 after the treatment. * $P < 0.05$, ** $P < 0.01$ compared to the sham-operated group on the same day. The data are expressed as the mean ± SEM for the indicated number of animals.

Fig. 2. Effects of MPTP on behavior in the object recognition test. (A) shows the percentage of time spent exploring object "B" or "D" during the test. (B) shows the rearing number during the test. Data are expressed as the mean ± SEM for the indicated number of animals. ** $P < 0.01$ compared to the sham-operated group at the same time point.

Fig. 3. Effects of MPTP on dopamine (DA)-containing neurons in the striatum and substantia nigra pars compacta (SNc). The brains were taken 28 days after MPTP treatment. DAergic neurons, stained by tyrosine hydroxylase (TH) immunoreaction, are shown in the representative coronal sections of the striatum (A, B) and SNc (C, D) (magnification, ×50; bar, 200 μm) in sham-operated and MPTP-treated rats. Black squares (24 585μm^2) in the schematic drawings are used for measuring optical density (OD) of TH immunoreactivity in the striatum and neuronal density (per mm^2) in the SNc. MPTP-treated rats show decreases in relative OD of TH immunoreactivity in the striatum (E) and in density of DAergic neurons in the SNc (F). * $P < 0.05$, ** $P < 0.01$, compared to

corresponding sham-operated rats. The schematic drawings are modified from the rat brain atlas (Paxinos and Watson, 1986).

Fig. 4. Effects of MPTP on microglial activation in the brain. The brains were taken 28 days after MPTP lesion. No activated microglia, OX-6-positive cells, was found in the substantia nigra pars compacta (SNc) and hippocampus (HIP) of sham-operated rats. MPTP treatment caused a massive accumulation of activated microglia in the SNc and HIP (magnification, ×50; bar, 200 μm). Insets show activated microglia at magnification of ×400, bar, 20μm.

Fig. 5. Effects of MPTP on neurons in the hippocampal CA1 area. The brains were taken 28 days after MPTP treatment. Images show Nissl-stained pyramidal neurons in the CA1 area of hippocampus, as indicated in the square of schematic drawing, in sham-operated (A) and MPTP-treated (B) rats (magnification, ×200; bar, 100 μm). MPTP-treated rats show decrease in neuronal area in the CA1 area, ** $p < 0.001$, compared to sham-operated rats (C).

Table 1.

Table 1. Effect of MPTP on behavior in the elevated plus-maze test on day 25.

	Sham (n=15)	MPTP (n=25)
Open arm latency (sec)	154.0 ± 36.9	242.0 ± 20.2*
Open arm time (sec)	37.4 ± 12.5	11.0 ± 4.6*
Enclosed arm time (sec)	191.7 ± 21.0	248.6 ± 10.2*
Open arm entries (no.)	4.1 ± 1.1	1.4 ± 0.5*
Enclosed arm entries (no.)	8.1 ± 1.4	5.9 ± 0.8
Total distance (cm)	2 270.8 ± 265.7	1 934.9 ± 192.7
Total rearing (no.)	16.9 ± 1.3	14.7 ± 1.0

* $p < 0.05$, compared to the sham-operated group. The data are expressed as the mean ± SEM for the indicated number of animals.

Table 2.

Table 2. Correlations between behavior in the elevated plus-maze and object recognition tests.

	Object recognition					
	Total exploratory time (sec)		% D exploration time		Rearing (no.)	
Elevated plus-maze	Pearson Correlation	*p* value	Pearson Correlation	*p* value	Pearson Correlation	*p* value
Open arm latency (sec)	-0.172	0.289	-0.03	0.856	-0.152	0.348
Open arm time (sec)	-0.072	0.661	0.321	0.043*	0.167	0.302
Enclosed arm time (sec)	-0.126	0.44	-0.39	0.013*	-0.33	0.037*
Open arm entries (no.)	-0.008	0.96	0.314	0.049*	0.104	0.523
Enclosed arm entries (no.)	0.284	0.076	0.177	0.275	-0.033	0.841
Total distance (cm)	0.171	0.403	0.108	0.601	-0.091	0.658
Total rearing (no.)	0.355	0.025	0.034	0.836	0.289	0.07

P values, 2-tailed.

Fig. 1.

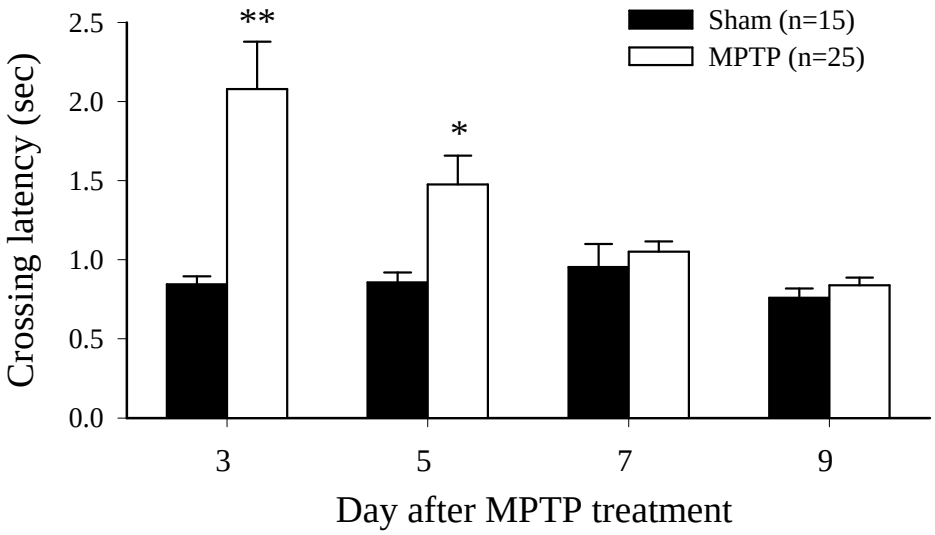

Crossing latency (sec)
2.5
2.0
1.5
1.0
0.5
0.0
**
*
Sham (n=15)
MPTP (n=25)
3
5
7
9
Day after MPTP treatment

Fig. 2.

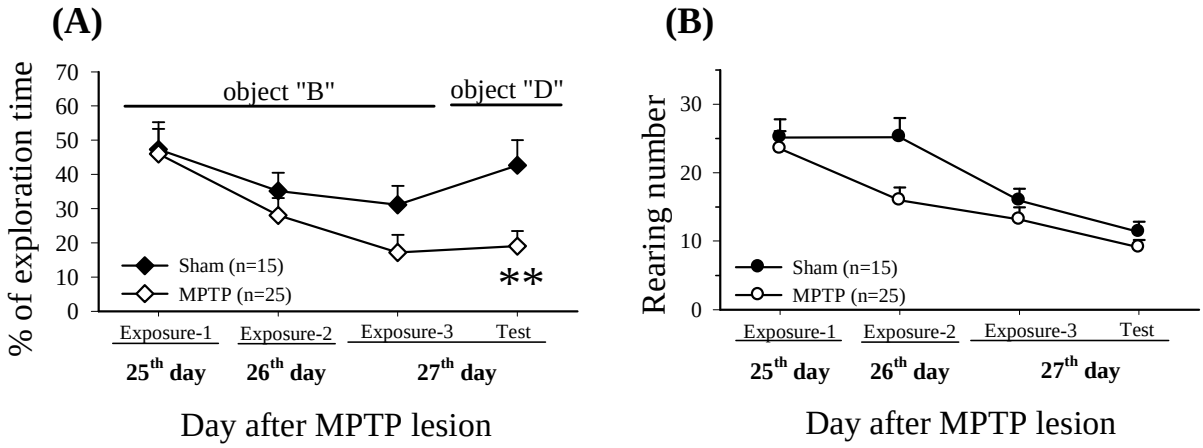

(A)
% of exploration time
70
60
50
40
30
20
10
0
object "B"
object "D"
Sham (n=15)
MPTP (n=25)
**
Exposure-1
Exposure-2
Exposure-3
Test
25th day
26th day
27th day
Day after MPTP lesion
(B)
Rearing number
30
20
10
0
Sham (n=15)
MPTP (n=25)
Exposure-1
Exposure-2
Exposure-3
Test
25th day
26th day
27th day
Day after MPTP lesion

Fig. 3.

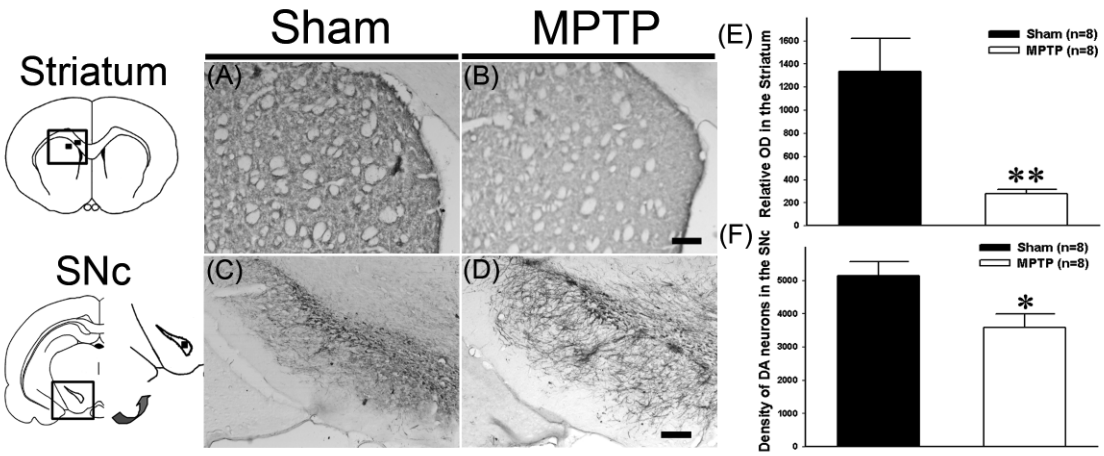

Sham
MPTP
Striatum
SNc
(A)
(B)
(C)
(D)
(E)
Relative OD in the Striatum
Sham (n=8)
MPTP (n=8)
**
(F)
Density of DA neurons in the SNc
Sham (n=8)
MPTP (n=8)
*

Fig. 4.

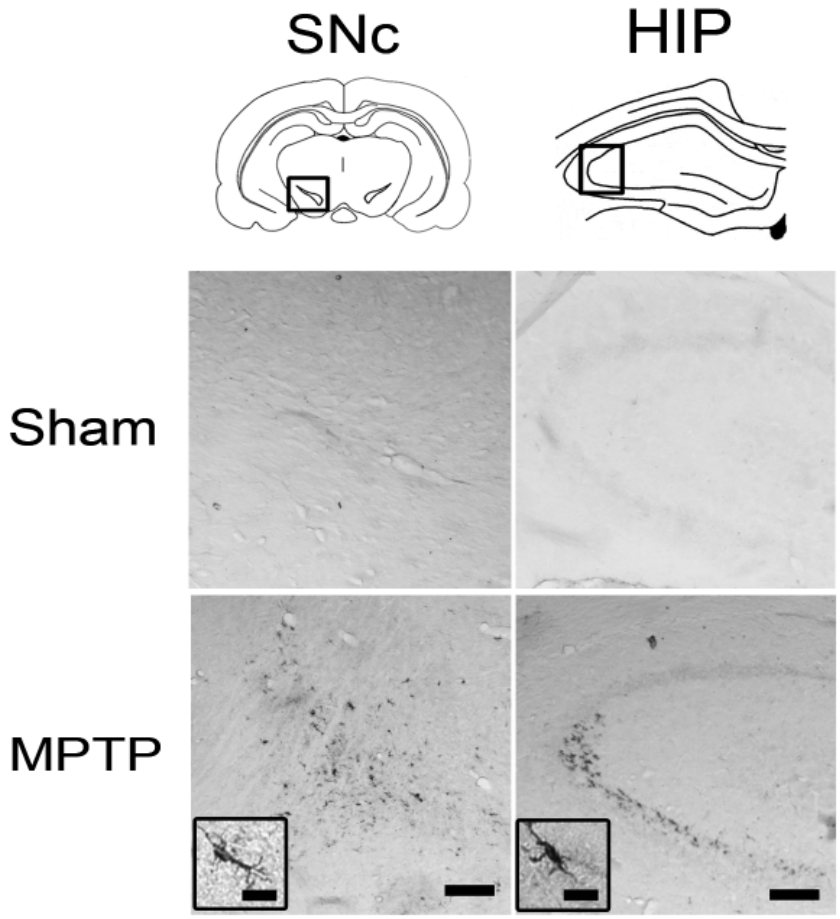
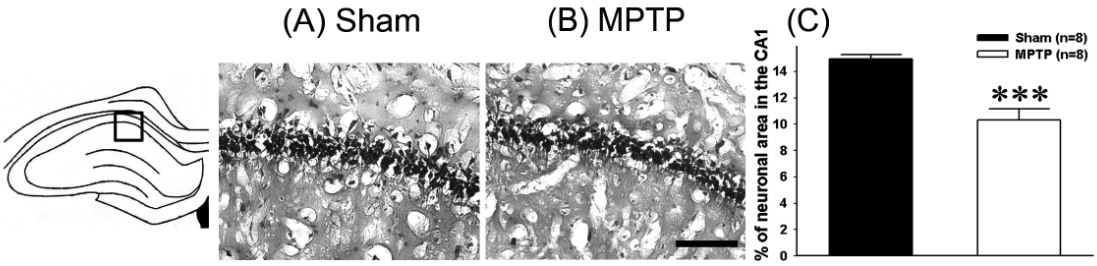

SNc
HIP
Sham
MPTP

Fig. 5.


(A) Sham
(B) MPTP
(C)
Sham (n=8)
MPTP (n=8)
***
% of neuronal area in the CA1
0
2
4
6
8
10
12
14