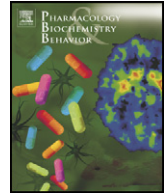




Contents lists available at ScienceDirect

Pharmacology, Biochemistry and Behavior

journal homepage: www.elsevier.com/locate/pharmbiochembeh

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

Hiu-Ngar Sy^{a,1}, Shey-Lin Wu^{a,b,1}, Wen-Fu Wang^a, Chang-Hao Chen^c, Yao-Ting Huang^a, Ying-Ming Liou^d, Chien-Shun Chiou^e, Cornelius Rainer Pawlak^{f,g,*}, Ying-Jui Ho^{c,*}

^a Department of Neurology, Chang-Hua Christian Hospital, Taiwan, ROC

^b Department of Bioindustry Technology, Dayeh University, Taiwan, ROC

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

^d Department of Life Sciences, National Chung Hsing University, Taiwan, ROC

^e The Central Branch Office, Center for Disease Control, Taiwan, ROC

^f Department of Cognitive and Clinical Neuroscience, Central Institute of Mental Health, Mannheim, Germany

^g Department of Psychology, Goethe-University, Frankfurt am Main, Germany

ARTICLE INFO

Article history:

Received 12 June 2009

Received in revised form 12 November 2009

Accepted 22 December 2009

Available online 11 January 2010

Keywords:

Parkinson's disease

Dementia

Object recognition

Neuroinflammation

Microglial activation

MPTP

ABSTRACT

Emotional changes, impairment of object recognition, and neuroinflammation are seen in Parkinson's disease with dementia (PDD). Here, we show 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, and cell loss in the hippocampal CA1 area. With regard to behavior, an increase in anxiety-like behavior and impairment of object recognition were observed during the fourth week after MPTP lesioning. 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 be involved in 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. MPTP-lesioned rats are therefore proposed as a useful tool for assessing the ability of pharmacological agents to prevent recognition deficits in PDD.

© 2010 Elsevier Inc. All rights reserved.

1. Introduction

Parkinson's disease (PD) is a highly prevalent neurodegenerative disorder (Aarsland et al., 2005). In addition to 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%, up to six times higher than in healthy people (Aarsland et al., 2001). Emotional changes (Levin et al., 1991), for example, increased anxiety levels (Fenelon et al., 2000), visuospatial dysfunctions (Crucian and Okun, 2003; Emre, 2003), and impairment of facial recognition and object discrimination (Barnes et al., 2003; Laatu et al., 2004; Ramirez-Ruiz

et al., 2006) are the main symptoms in Parkinson's disease with dementia (PDD).

Although loss of dopamine (DA)-containing neurons in the substantia nigra is the main characteristic of PD (McGeer and McGeer, 2004), inflammation has also been proposed as a possible mechanism in the pathogenesis, as considerably greater inflammation is seen in the substantia nigra in PD patients compared to controls (Abramsky and Litvin, 1978; McGeer and McGeer, 2004). Nigrostriatal dysfunction alone is probably not sufficient for the development of dementia in PD, as activated microglia have been observed not only in the substantia nigra and putamen, where DA loss is prominent, but also in the hippocampus of patients with PD (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 in 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.

* Corresponding authors. Ho is to be contacted at School of Psychology, Chung Shan Medical University, No. 110, Sec. 1, Jiaunguo N. Rd., Taichung City 402, Taiwan, ROC. Tel.: +886 4 24730022x11858; fax: +886 4 23248191. Pawlak, Department of Psychology, Goethe-University, Frankfurt am Main, J5, D-68159 Mannheim, Germany. Tel.: +49 621 1703 6262; fax: +49 621 1703 6255.

E-mail addresses: cornelius.pawlak@zi-mannheim.de (C.R. Pawlak), yjho@csmu.edu.tw, joshuayjho@yahoo.com.tw (Y.-J. Ho).

¹ Equal contribution.

Animals treated with 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) are widely used as an animal model of PD, as MPTP selectively destroys the DAergic system (Langston et al., 1983; Langston et al., 1999) and causes microglial activation in the brain of humans (Mogi et al., 1996). Deficits in habit learning and spatial memory in the active avoidance test (Da Cunha et al., 2001) and in the water maze test (Da Cunha et al., 2002; Da Cunha et al., 2003) have been reported in an MPTP-lesioned rat PD model. Disturbance of visual discrimination in MPTP-lesioned monkeys has also been described (Schneider et al., 2000). However, no data are available for object recognition in MPTP-lesioned rodents, which would be a better model for the disability in object recognition in PDD. To obtain a better understanding of the pathophysiology of PDD, it is necessary to detect neuronal changes occurring after degeneration of the nigrostriatal DAergic system. 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, the relationships between MPTP-induced microglial activation and behavioral deficiencies (Da Cunha et al., 2001; Ferro et al., 2005) are not yet understood. In this study, we examined motor and emotional behavior and object recognition in rats after MPTP lesioning using a battery of behavioral tests, namely a bar test as a measure of catalepsy (Sanberg et al., 1988), an elevated plus-maze test 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 the MPTP-induced DAergic degeneration and deficits in emotional behavior and object recognition were accompanied by neuroinflammation in the hippocampus. It is therefore 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 ($35 \times 56 \times 19$ 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 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 or vehicle on 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). Because it has been demonstrated that high-intensity illumination is an aversive stimulus that decreases exploratory behavior in rats in an unfamiliar environment (Garcia et al., 2005), all behavioral experiments were performed under dim illumination with red light (28 lx), as in our previous studies (Wang et al., 2009; Wu et al., 2008). Before each test trial, the test equipment and object were cleaned using 20% ethanol and thoroughly dried. On day 28 after MPTP lesioning, the rats were sacrificed by exposure to CO₂ and transcardially perfused with phosphate-buffered saline (PBS), followed by 4% paraformaldehyde in PBS. The brains were immediately removed and post-fixed at 4 °C in 20% sucrose solution containing 4% paraformaldehyde for histochemical assessment.

2.3. Surgery

Brain surgery was performed on a stereotaxic instrument. The rats received an 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 (Sigma, MO, USA) ($1 \mu\text{mol}$ in $2 \mu\text{l}$ of saline) was bilaterally infused into the SNc through a 30 gauge stainless needle at a rate of $0.7 \mu\text{l}/\text{min}$ 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. In parallel, sham-operated rats ($n = 15$) were subjected to the same procedure, but were infused with $2 \mu\text{l}$ of saline instead of MPTP. Immediately after surgery, the rats received an intramuscular injection of penicillin-G procaine (0.2 ml, 20,000 IU) and were housed individually in plastic cages ($25 \times 41 \times 19$ cm) for 10 days, then were regrouped 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 tests were monitored and scored 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 greater than 14 pictures/s.

2.4.1. Bar test

The bar test was performed on days 3, 5, 7, and 9 after MPTP lesioning. Catalepsy was measured as the mean time taken for 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.

2.4.2. Elevated plus-maze test

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

2.4.3. Object recognition test

The apparatus and testing procedure for the object recognition test were similar to those described previously (Mumby et al., 2005; Wang et al., 2009; Wu et al., 2008). An open field arena was constructed of black polyvinyl plastic (100 cm long \times 100 cm wide \times 60 cm high). Each rat was subjected to 3 exposure sessions at 24 h intervals, then, 5 min after the last exposure session, a test session was performed. Four different objects made of transparent glass, paper, porcelain, or metal (all about $10 \times 10 \times 10$ 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 this was repeated 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 rearing number during the exposure and test sessions were recorded. The percentage of the exploration time spent on object

B or D in the sessions $[(\text{Time}_B \text{ or } D / \text{Time}_{\text{all objects}}) \times 100\%]$ 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 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) (1:4000; 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 then incubated sequentially for 30 min at 37 °C 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.

2.5.1. Image analysis

The stained brain sections, identified according to the rat brain atlas (Paxinos and Watson, 1986), were used to measure histological changes using methods described previously (Xavier et al., 2005) and a microscope (ZEISS AXioskop2, Germany) coupled to a CCD (Optronics, USA) and Image Pro Plus Software 4.1 (Media Cybernetics, CA, USA). In this study, areas of interest measuring 24 585 μm^2 were created to determine the optical density of TH immunoreactivity in the striatum and the neuronal density in the SNc. Other areas of interest measuring 40 000 μm^2 were created to estimate the neuronal density in the hippocampal CA1 area. To measure the intensity of DAergic projections in the striatum, the TH-stained images were converted to gray scale, then the gray level of the area of interest was measured and the background staining, measured in the non-immunoreactive corpus callosum, was subtracted. Thus, the relative optical density refers to the values generated by the TH-reactive tissue. To measure the density of DAergic neurons in the SNc, the images were captured and not converted to gray scale, and an area of interest was overlaid in this region and the somas of TH-immunoreactive neurons in this area 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, so a semi-quantitative method, the area of Nissl-stained neurons expressed as a percentage of an area of interest in the CA1 area, was used to represent the neuronal density.

2.6. Data analysis

Analysis of variance (ANOVA) followed by Scheffé's post hoc test was used to analyze the data for the elevated plus-maze and bar tests. ANOVA repeated measures were used to analyze the data for the object recognition test. Pearson's coefficient was used to evaluate the relationships between the behavior in the elevated plus-maze and object recognition tests. Student's *t* test was used to analyze the histological data. The behavioral data from all of the rats were used for analysis. Eight randomly selected brains per group were used for histological assay. All results are expressed as the mean \pm SEM. The level of significance was defined as $P < 0.05$ (two-tailed).

3. Results

3.1. Behavior

After MPTP lesioning, 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 \times lesion interaction ($F(3,114) = 6.66, P < 0.001$). Significant longer crossing latencies in the MPTP-lesioned rats were seen on days 3 and 5 after MPTP lesioning ($df = 38, t\text{-values} \geq 2.54, P\text{-values} \leq 0.05$), but not on days 7 and 9 (Fig. 1).

An increased open arm latency, decreased open arm time, and fewer open arm entries in the elevated plus-maze test compared to the controls were seen in MPTP-lesioned rats on day 25 ($df = 38, t\text{-values} > 1.98, P\text{-values} < 0.05$), indicating higher anxiety-like levels. However, there was no difference between the groups in the rearing number, enclosed arm entries, and total distance traveled by the rats in the elevated plus-maze test, which are commonly used to measure general activity (Table 1).

Fig. 2 shows the recognition and rearing behavior of rats in the object recognition test on days 25–27. MPTP-lesioned rats spent a lower percentage of time exploring the novel object “D” in the object recognition test on day 27 after MPTP lesioning than the sham-operated controls ($df = 38, t = 2.94, P \leq 0.01$) (Fig. 2A). As shown in Fig. 2, ANOVA with repeated measures revealed a significant time effect ($F(3,114) = 24.86, P \leq 0.001$) for rearing number in the object recognition test, but no significant lesion effect or time \times lesion interaction.

The open arm time and number of open arm entries in the elevated plus-maze test were significantly correlated with the percentage of time spent in exploring novel object “D” in the object recognition test ($P\text{-values} \leq 0.05$). However, the enclosed arm time was negatively correlated with the percentage of time spent in exploring novel object “D” and the rearing number ($P\text{-values} \leq 0.05$) (Table 2).

3.2. 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 in the sham-operated and MPTP-lesioned groups. TH immunoreactivity was observed in neuronal cell bodies and their processes in both groups. MPTP lesioning resulted in a significant reduction in the relative optical density of TH immunoreactivity in the striatum ($df = 14, t = 3.71, P < 0.01$) and in the 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

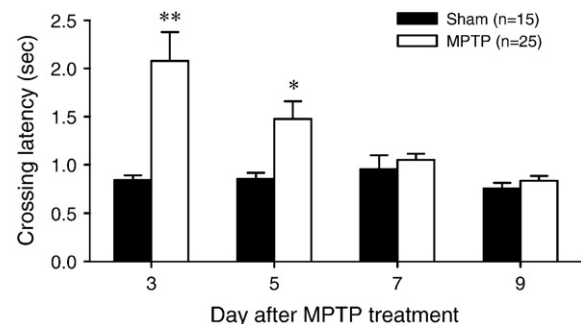


Fig. 1. Effect of MPTP on behavior in the bar test. MPTP was bilaterally infused into the substantia nigra pars compacta (SNc) on day 0, then the catalepsy bar test was performed on days 3, 5, 7, and 9. * $P < 0.05$, ** $P < 0.01$ compared to the sham-operated group on the same day. The data are expressed as the mean \pm SEM for the indicated number of animals.

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 (s)	154.0 ± 36.9	242.0 ± 20.2*
Open arm time (s)	37.4 ± 12.5	11.0 ± 4.6*
Enclosed arm time (s)	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

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

cells, was evident in the SNc and hippocampus (Fig. 4), but not in the prefrontal cortex, striatum, or cerebral cortex (data not shown) of the MPTP-lesioned group, but not the controls. Semi-quantitative analysis confirmed that MPTP lesioning resulted in a significant decrease in 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).

4. Discussion

Four weeks after intra-SNc infusion of MPTP, neurodegeneration of the nigrostriatal DAergic system, cell loss in the hippocampal CA1 area, and microglial activation in the SNc and hippocampus were seen. Furthermore, emotional-related and cognitive changes were also observed during the fourth week after MPTP lesioning, namely, an increase in anxiety-like avoidance behavior and impairment of object recognition. We did not find evidence of motor impairment 7 days after MPTP lesioning, a time at which catalepsy was no longer

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

	Object recognition					
	Total exploratory time (s)		% D exploration time		Rearing (no.)	
Elevated plus-maze	Pearson correlation	P value	Pearson correlation	P value	Pearson correlation	P value
Open arm latency (s)	-0.172	0.289	-0.03	0.856	-0.152	0.348
Open arm time (s)	-0.072	0.661	0.321	0.043*	0.167	0.302
Enclosed arm time (s)	-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.
* $P < 0.05$.

observed, suggesting that the behavioral differences seen in the 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 the DAergic degeneration-related behavioral deficits.

MPTP is a toxin commonly used to induce an animal model of PD, as it causes specific degeneration of DAergic neurons in the substantia nigra and loss of nerve terminals in the striatum (Langston et al., 1983; Langston et al., 1999). In the present study, intra-SNc infusion of MPTP resulted in a significant decrease in TH immunohistochemical staining in the SNc and striatum, indicating lesions in the nigrostriatal DAergic system. Although a stereological approach involving the counting of cells in a complete series of sections would provide additional data (Ferro et al., 2005; Meissner et al., 2003), calculating the cell number in representative brain sections yielded similar histological results to those reported in the literature (Da Cunha et al., 2002; Da Cunha et al., 2001). MPTP-lesioned rats have been reported to show lower spontaneous locomotion than controls in an open field test one day after surgery (Capitelli et al., 2008; Perry et al., 2005; Reksidler et al., 2008), but the behavioral changes were no longer observed on day 7 (Capitelli et al., 2008) or day 18 (Ferro et al., 2005; Perry et al., 2005). Furthermore, in line with previous reports showing transitory catalepsy 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 lesioning. Recovery of motor function was further supported by the lack of any differences between the groups in terms of rearing number, enclosed arm entries, and distance traveled by the rats in the elevated plus-maze test and rearing number in the object recognition test, indicating an absence of gross motor impairment and suggesting that the behavioral performance in the tests was not confounded by motor impairment. One interesting point is whether the impairment of object recognition occurred during the acute phase of MPTP lesioning, but this cannot be answered, as the animals show motor disturbance during the acute phase after MPTP lesioning.

Similar behavioral deficits are seen in patients with PDD and MPTP-lesioned rats. Consistent with a report of high anxiety levels in the late course of PD (Fenelon et al., 2000), MPTP lesioning caused an increase in anxiety-like behavior in the elevated plus-maze test, indicated as a reduced open arm time and increased enclosed arm time. In addition, the dysfunction of object recognition seen in the MPTP-lesioned rats may be compatible with the visuospatial (Girotti et al., 1988), facial recognition, and object discrimination deficits (Barnes et al., 2003; Ramirez-Ruiz et al., 2006) reported in PD patients. Interestingly,

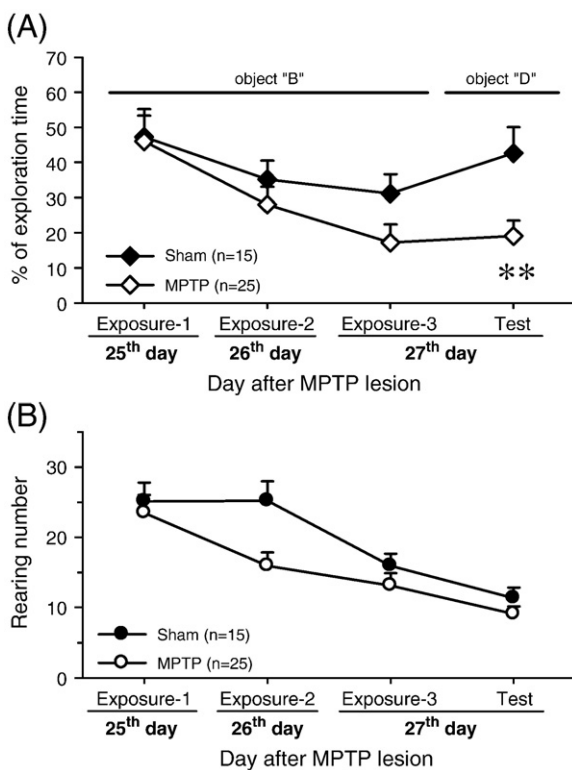


Fig. 2. Effect of MPTP on behavior in the object recognition test. (A) Percentage of time spent exploring object "B" or "D" during the test. (B) Rearing number during the test. Data are expressed as the mean ± SEM for the indicated number of animals. ** $P < 0.01$ compared to the sham-operated group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

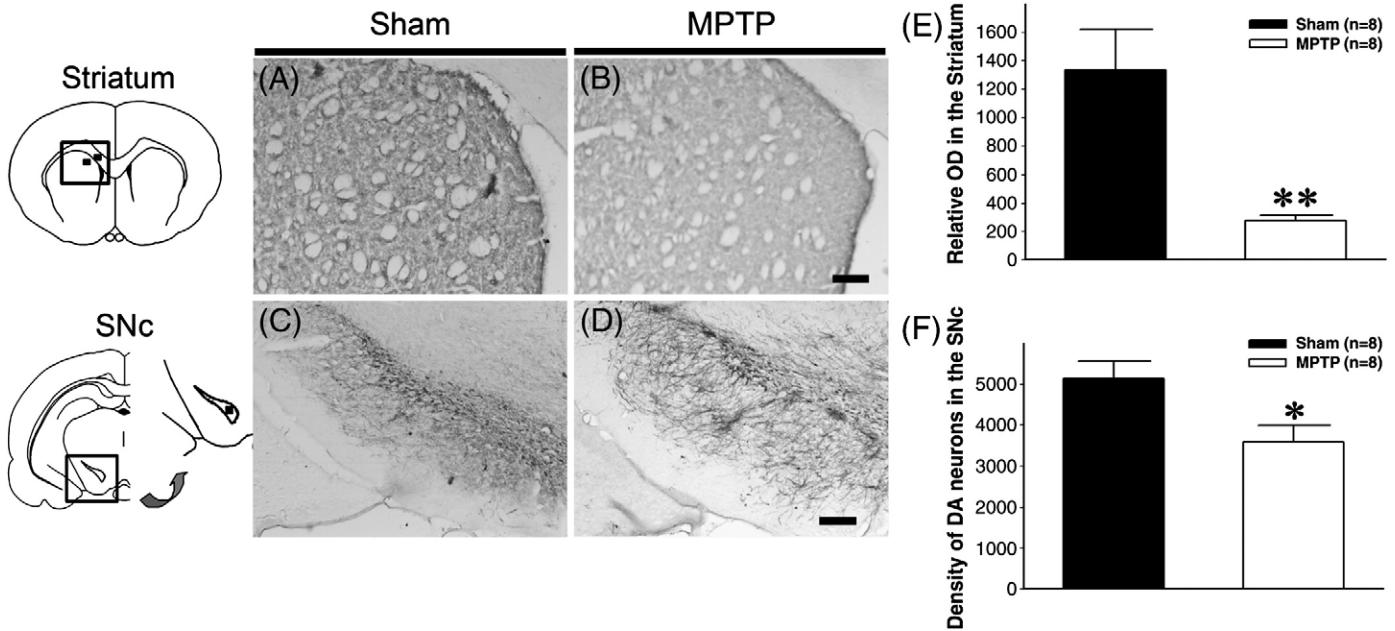


Fig. 3. Effect of MPTP on dopamine (DA)-containing neurons in the striatum and substantia nigra pars compacta (SNc). The brains were taken 28 days after MPTP lesioning. DAergic neurons, stained for tyrosine hydroxylase (TH), are shown in representative coronal sections of the striatum (A, B) and SNc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

anxiety levels were negatively correlated with the time spent exploring a novel object, but not correlated with total exploration time. This result is consistent with the findings that cognition-impaired 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 either healthy controls or cognitively preserved PD patients in object recognition tests in which the subjects used visual sensation for the object recognition task, as they had to respond to a stimulus (pictures or written words) presented on a screen (Laatu et al., 2004). Moreover, using a battery of visual object and space perception tests, Barnes et al. (2003) demonstrated that patients with PDD, even those with adequate eyesight, exhibit impairment in tests of object recognition (impaired face recognition and silhouette identification). In the present study, the MPTP-lesioned rats also showed deficits in object recognition. As in humans, such deficits may not be due to dysfunction of visual perception and, in addition, the rats may not rely solely on visual sensations for the test. Thus, similar mechanisms may underlie the object recognition impairments seen in patients with PDD and MPTP-lesioned rats. Furthermore, it has been demonstrated that MPTP-treated rats show deficits in spatial working memory performance 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). These results suggest that MPTP-lesioned rats may be a good model for PDD.

The hippocampus is involved in 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). Hippocampal lesions in rats impair both spatial learning (Gilbert and Kesner, 2006) and episodic-like memory integrating “what,” “where,” and “when” elements (Li and Chao, 2008). Moreover, the hippocampal CA1 area is responsible for temporal memory (Hunsaker et al., 2006). Lesions in this region correspond to, and probably account for, lower scores in the object recognition test in rats (Hunsaker et al., 2008). Moreover, ischemia-induced cell loss in the hippocampal CA1 area of rats produces object recognition deficits in acquisition and retention in a delayed nonmatching-to-sample task (Wood et al., 1993). In addition, microglial activation in

the hippocampal CA1 area leads to learning and memory deficits in rats (Tanaka et al., 2006). Local pharmacological manipulations in the CA1 area also affect consolidation of object recognition memory (Clarke et al., 2008; de Lima et al., 2006). The present study showed that MPTP lesioning resulted in object recognition deficits that were accompanied by both microglial activation in the hippocampus (indicated by massive accumulation of OX-6-positive cells) (Ogura et al., 1994) and a decrease in the density of pyramidal neurons in the hippocampal CA1 area. These data may indicate that hippocampal CA1 area is a possible neuronal substrate involved in DAergic degeneration-related impairment of object recognition.

Although the 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 patients with PDD are strongly correlated 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, leads 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 patients with PD (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 in dementia with Lewy bodies (Imamura et al., 2005). In addition, glutamatergic N-methyl-D-aspartate (NMDA) receptors in the hippocampus play a crucial role in learning, memory, and recognition (Baker and Kim, 2002; Costa et al., 2008; Hammond et al., 2004; Rampon et al., 2000; Shi et al., 2006). However, hyperactivation of NMDA receptors results in excitotoxicity (Liu et al., 2007) and the consequences of microglial activation (Bernal et al., 2000). Activated microglia can, in turn, facilitate the over-activation of NMDA receptors by increased glutamatergic transmission (Bezzi et al., 2001)

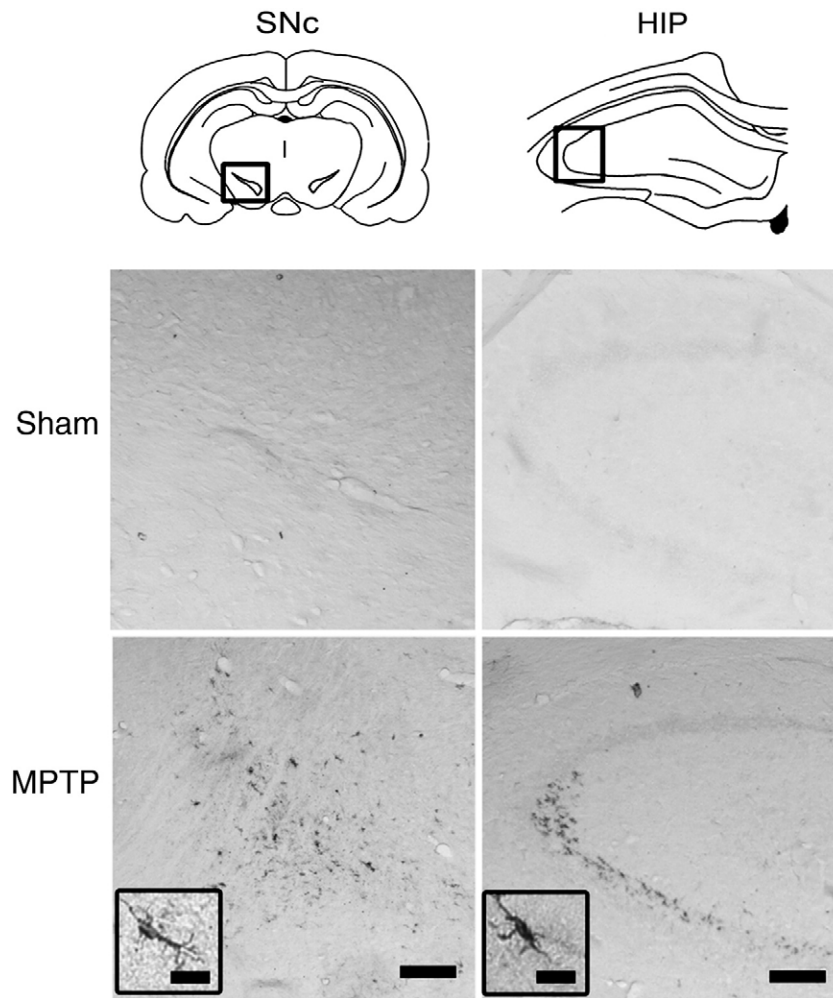


Fig. 4. Effect of MPTP on microglial activation in the brain. The brains were taken 28 days after MPTP lesioning and activated microglia immunostained with antibodies against rat MHC class II (OX-6) in the substantia nigra pars compacta (SNc) and hippocampus (HIP). Magnification, $\times 50$; bar, 200 μm . The insets show activated microglia at a magnification of $\times 400$, bar, 20 μm .

and release of inflammatory cytokines (Jara et al., 2007). Infusion of lipopolysaccharide into the rat brain causes microglial activation and impairment of hippocampal-dependent memory; and these effects are reduced by memantine, an antagonist of NMDA receptors (Rosi et al., 2006). Furthermore, our recent study showed that recovery of episodic-like memory after treatment with D-cycloserine, a partial agonist of NMDA receptors, is accompanied by suppression of MPTP-induced microglial activation and cell loss in the hippocampal CA1 area

(Wang et al., 2010). These results suggest that glutamatergic hyperactivity may be involved in the MPTP-induced neuroinflammation and cell loss in the hippocampus and the sequela of recognition impairments. However, the links between the degeneration of DAergic neurons and dysfunction of the glutamatergic system require further study. Non-steroidal anti-inflammatory drugs are able to reduce the neuronal death induced by activated microglia and improve motor impairment in PD (Hirohata et al., 2008; Klegeris and McGeer, 2005).

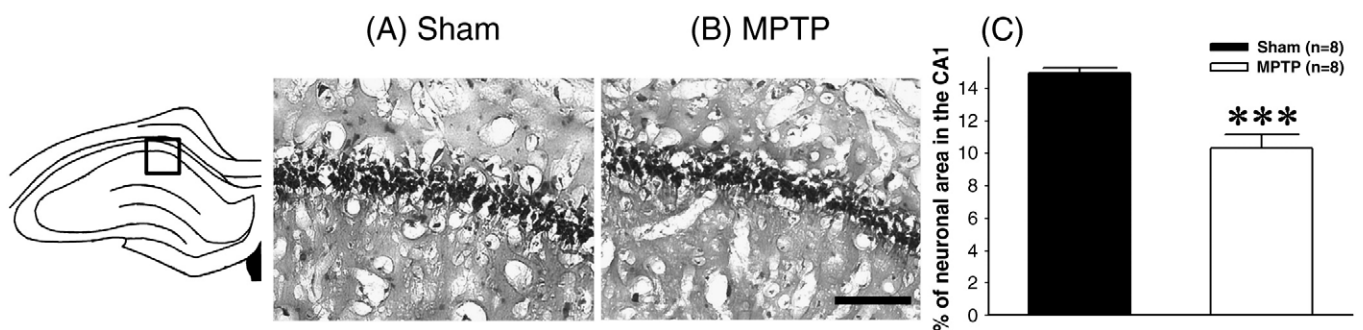


Fig. 5. Effect of MPTP on neurons in the hippocampal CA1 area. The brains were taken 28 days after MPTP lesioning. The images show Nissl-stained pyramidal neurons in the CA1 area of hippocampus, as indicated in the square in the schematic drawing, in sham-operated (A) and MPTP-lesioned (B) rats (magnification, $\times 200$; bar, 100 μm). *** $P < 0.001$, compared to sham-operated rats (C).

Thus, suppression of microglial activation using anti-inflammatory compounds may be a feasible strategy for preventing neuronal and cognitive decline in PD (Sriram et al., 2006).

In addition to DAergic degeneration in the SNc and striatum, consistent with the pathophysiology of PD, intra-nigral infusion of MPTP also caused increased microglial activation in the brain and cell loss in the hippocampal CA1 area, emotional changes, and object recognition deficits. These findings show that MPTP-induced neuroinflammation and its consequences may be involved in 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. MPTP-lesioned rats are therefore proposed as a useful tool for assessing the ability of pharmacological agents to prevent recognition deficits in PDD.

Acknowledgments

The authors thank Drs. Yuan-Feen Tsai and Rainer K. Schwarting for their thoughtful comments on this manuscript, Dr. Ren-Hau Li for statistical advice, Dr. Jay-Shake Li and Mr. Sheng-Yuan Wang for kindly providing their self-developed software to collect the behavioral data, and Ms. An-Li Wang and Shih-Chun Ho for their technical assistance. This work was supported by grants from the National Science Council of the ROC (NSC 96-2320-B-040-019; NSC 98-2410-H-040-003; DAAD/NSC Project Based Personnel Exchange Program 0950042882P), the Chang-Hua Christian Hospital (CCH grant 93118 and 92137), the Chung Shan Medical University (097-CCH-CSMU-14), and the 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;56:730–6.
- Aarsland D, Zaccari J, Brayne C. A systematic review of prevalence studies of dementia in Parkinson's disease. *Mov Disord* 2005;20:1255–63.
- Abramsky O, Litvin Y. Autoimmune response to dopamine-receptor as a possible mechanism in the pathogenesis of Parkinson's disease and schizophrenia. *Perspect Biol Med* 1978;22:104–14.
- Baker KB, Kim JJ. Effects of stress and hippocampal NMDA receptor antagonism on recognition memory in rats. *Learn Mem* 2002;9:58–65.
- Barnes J, Boubert L, Harris J, Lee A, David AS. Reality monitoring and visual hallucinations in Parkinson's disease. *Neuropsychologia* 2003;41:565–74.
- Bernal F, Saura J, Ojuel J, Mahy N. Differential vulnerability of hippocampus, basal ganglia, and prefrontal cortex to long-term NMDA excitotoxicity. *Exp Neurol* 2000;161:686–95.
- Bezzi P, Domercq M, Brambilla L, Galli R, Schols D, De Clercq E, et al. CXCR4-activated astrocyte glutamate release via TNF α : amplification by microglia triggers neurotoxicity. *Nat Neurosci* 2001;4:702–10.
- Broadbent NJ, Squire LR, Clark RE. Spatial memory, recognition memory, and the hippocampus. *Proc Natl Acad Sci U S A* 2004;101:14515–20.
- Brown RG, Marsden CD. How common is dementia in Parkinson's disease? *Lancet* 1984;2:1262–5.
- Capitelli C, Sereniki A, Lima MM, Reksidler AB, Tufik S, Vital MA. Melatonin attenuates tyrosine hydroxylase loss and hypolocomotion in MPTP-lesioned rats. *Eur J Pharmacol* 2008;594:101–8.
- Caraceni A, Martini C, Belli F, Mascheroni L, Rivoltini L, Arienti F, et al. Neuropsychological and neurophysiological assessment of the central effects of interleukin-2 administration. *Eur J Cancer* 1993;29A:1266–9.
- Clarke JR, Rossato JJ, Monteiro S, Bevilacqua 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;90:374–81.
- Costa MS, Botton PH, Mioranza S, Ardaes AP, Moreira JD, Souza DO, et al. Caffeine improves adult mice performance in the object recognition task and increases BDNF and TrkB independent on phospho-CREB immunoccontent in the hippocampus. *Neurochem Int* 2008;53:89–94.
- Crucian GP, Okun MS. Visual-spatial ability in Parkinson's disease. *Front Biosci* 2003;8:s92–7.
- 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, 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;22:227–37.
- Da Cunha C, Wietzikoski S, Wietzikoski EC, Miyoshi E, Ferro MM, Anselmo-Franci JA, et al. Evidence for the substantia nigra pars compacta as an essential component of a memory system independent of the hippocampal memory system. *Neurobiol Learn Mem* 2003;79:236–42.
- 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;405:142–6.
- Denicoff KD, Rubino 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;14:1189–97.
- Emre M. Dementia associated with Parkinson's disease. *Lancet Neurol* 2003;2:229–37.
- Fenelon G, Mahieux F, Huon R, Ziegler M. Hallucinations in Parkinson's disease: prevalence, phenomenology and risk factors. *Brain* 2000;123(Pt 4):733–45.
- Ferro MM, Bellissimo MI, Anselmo-Franci JA, Angelucci ME, Canteras NS, Da Cunha C. Comparison of bilaterally 6-OHDA- and MPTP-lesioned rats as models of the early phase of Parkinson's disease: histological, neurochemical, motor and memory alterations. *J Neurosci Methods* 2005;148:78–87.
- Garcia AM, Cardenas FP, Morato S. Effect of different illumination levels on rat behavior in the elevated plus-maze. *Physiol Behav* 2005;85:265–70.
- Gilbert PE, Kesner RP. The role of the dorsal CA3 hippocampal subregion in spatial working memory and pattern separation. *Behav Brain Res* 2006;169:142–9.
- Girotti F, Soliveri P, Carella F, Piccolo I, Caffarra P, Musicco M, et al. Dementia and cognitive impairment in Parkinson's disease. *J Neurol Neurosurg Psychiatr* 1988;51:1498–502.
- Hammond RS, Tull LE, Stackman RW. On the delay-dependent involvement of the hippocampus in object recognition memory. *Neurobiol Learn Mem* 2004;82:26–34.
- 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;54:620–7.
- 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;1043:179–85.
- Hunsaker MR, Thorup JA, Welch T, Kesner RP. The role of CA3 and CA1 in the acquisition of an object-trace-place paired-associate task. *Behav Neurosci* 2006;120:1252–6.
- Hunsaker MR, Lee B, Kesner RP. Evaluating the temporal context of episodic memory: the role of CA3 and CA1. *Behav Brain Res* 2008;188:310–5.
- Imamura K, Hishikawa N, Sawada M, Nagatsu T, Yoshida M, Hashizume Y. Distribution of major histocompatibility complex class II-positive microglia and cytokine profile of Parkinson's disease brains. *Acta Neuropathol* 2003;106:518–26.
- 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;109:141–50.
- Jara JH, Singh BB, Floden AM, Combs CK. Tumor necrosis factor alpha stimulates NMDA receptor activity in mouse cortical neurons resulting in ERK-dependent death. *J Neurochem* 2007;100:1407–20.
- Kadiu I, Glanzner 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;2:355–65.
- Laatu S, Revonsuo A, Pihko L, Portin R, Rinne JO. Visual object recognition deficits in early Parkinson's disease. *Parkinsonism Relat Disord* 2004;10:227–33.
- Langston JW, Ballard P, Tetrud JW, Irwin I. Chronic Parkinsonism in humans due to a product of meperidine-analog synthesis. *Science* 1983;219:979–80.
- Langston JW, Forno LS, Tetrud J, Reeves AG, Kaplan JA, Karluk D. Evidence of active nerve cell degeneration in the substantia nigra of humans years after 1-methyl-4-phenyl-1, 2, 3, 6-tetrahydropyridine exposure. *Ann Neurol* 1999;46:598–605.
- Levin BE, Llabre MM, Reisman S, Weiner WJ, Sanchez-Ramos J, Singer C, et al. Visuospatial impairment in Parkinson's disease. *Neurology* 1991;41:365–9.
- Levy G, Tang MX, Louis ED, Cote LJ, Alfaró B, Mejia H, et al. The association of incident dementia with mortality in PD. *Neurology* 2002;59:1708–13.
- Lewis SJ, Slobosz 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;43:823–32.
- Li JS, Chao YS. Electrolytic lesions of dorsal CA3 impair episodic-like memory in rats. *Neurobiol Learn Mem* 2008;89:192–8.
- Liu Y, Wong TP, Aarts M, Rooyakkers A, Liu L, Lai TW, et al. NMDA receptor subunits have differential roles in mediating excitotoxic neuronal death both in vitro and in vivo. *J Neurosci* 2007;27:2846–57.
- 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.
- McGeer PL, McGeer EG. Inflammation and neurodegeneration in Parkinson's disease. *Parkinsonism Relat Disord* 2004;10(Suppl 1):S3–7.
- 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;38:1285–91.
- Meissner W, Prunier C, Guilloleau D, Chalou S, Gross CE, Bezard E. Time-course of nigrostriatal degeneration in a progressive MPTP-lesioned macaque model of Parkinson's disease. *Mol Neurobiol* 2003;28:209–18.
- Mogi M, Harada M, Narabayashi H, Inagaki H, Minami M, Nagatsu T. Interleukin (IL)-1 beta, IL-2, IL-4, IL-6 and transforming growth factor-alpha levels are elevated in ventricular cerebrospinal fluid in juvenile parkinsonism and Parkinson's disease. *Neurosci Lett* 1996;211:13–6.
- Mumby DG, Tremblay A, Lecluse V, Lehmann H. Hippocampal damage and anterograde object-recognition in rats after long retention intervals. *Hippocampus* 2005;15:1050–6.

- Ogura K, Ogawa M, Yoshida M. Effects of ageing on microglia in the normal rat brain: immunohistochemical observations. *NeuroReport* 1994;5:1224–6.
- Owen AM, Sahakian BJ, Hodges JR, Summers BA, Polkey CE, Robbins TW. Dopamine-dependent frontostriatal planning deficits in early Parkinson's disease. *Neuropsychology* 1995;9:126–40.
- 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;195:322–9.
- Piekema C, Kessels RP, Mars RB, Petersson KM, Fernandez G. The right hippocampus participates in short-term memory maintenance of object-location associations. *Neuroimage* 2006;33:374–82.
- Ramirez-Ruiz B, Junque C, Marti MJ, Valldeoriola F, Tolosa E. Neuropsychological deficits in Parkinson's disease patients with visual hallucinations. *Mov Disord* 2006;21:1483–7.
- Rampon C, Tang YP, Goodhouse J, Shimizu E, Kyin M, Tsien JZ. Enrichment induces structural changes and recovery from nonspatial memory deficits in CA1 NMDAR1-knockout mice. *Nat Neurosci* 2000;3:238–44.
- 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;167:268–77.
- Rosi S, Vazdarjanova A, Ramirez-Amaya V, Worley PF, Barnes CA, Wenk GL. Memantine protects against LPS-induced neuroinflammation, restores behaviorally-induced gene expression and spatial learning in the rat. *Neuroscience* 2006;142:1303–15.
- Sanberg PR, Bunsey MD, Giordano M, Norman AB. The catalepsy test: its ups and downs. *Behav Neurosci* 1988;102:748–59.
- Sawada M, Imamura K, Nagatsu T. Role of cytokines in inflammatory process in Parkinson's disease. *J Neural Transm Suppl* 2006;373–81.
- 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;860:190–4.
- Sedelis M, Schwarting RK, Huston JP. Behavioral phenotyping of the MPTP mouse model of Parkinson's disease. *Behav Brain Res* 2001;125:109–25.
- Shi L, Adams MM, Long A, Carter CC, Bennett C, Sonntag WE, et al. Spatial learning and memory deficits after whole-brain irradiation are associated with changes in NMDA receptor subunits in the hippocampus. *Radiat Res* 2006;166:892–9.
- Sriram K, Matheson JM, Benkovic SA, Miller DB, Luster MI, O'Callaghan JP. Deficiency of TNF receptors suppresses microglial activation and alters the susceptibility of brain regions to MPTP-induced neurotoxicity: role of TNF-alpha. *FASEB J* 2006;20:670–82.
- Stern Y, Tetrad JW, Martin WR, Kutner SJ, Langston JW. Cognitive change following MPTP exposure. *Neurology* 1990;40:261–4.
- Tanaka S, Ide M, Shibutani T, Ohtaki H, Numazawa S, Shioda S, et al. Lipopolysaccharide-induced microglial activation induces learning and memory deficits without neuronal cell death in rats. *J Neurosci Res* 2006;83:557–66.
- Wang AL, Liou YM, Pawlak CR, Ho YJ. Involvement of NMDA receptors in both MPTP-induced neuroinflammation and deficits in episodic-like memory in Wistar rats. *Behavior Brain Res* 2010;208:38–46.
- Wang WF, Wu SL, Liou YM, Wang AL, Pawlak CR, Ho YJ. MPTP lesion causes neuroinflammation and deficits in object recognition in Wistar rats. *Behav. Neurosci.* 2009;123:1261–70.
- 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.
- Wu SL, Hsu LS, Tu WT, Wang WF, Huang YT, Pawlak CR, et al. Effects of D-cycloserine on the behavior and ERK activity in the amygdala: role of individual anxiety levels. *Behav Brain Res* 2008;187:246–53.
- Xavier LL, Viola GG, Ferraz AC, Da Cunha C, Deonizio JM, Netto CA, et al. A simple and fast densitometric method for the analysis of tyrosine hydroxylase immunoreactivity in the substantia nigra pars compacta and in the ventral tegmental area. *Brain Res Protoc* 2005;16:58–64.
- 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.