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MPTP Lesion Causes Neuroinflammation and Deficits in Object Recognition in Wistar Rats

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Animal models of Parkinson's disease with dementia would greatly facilitate research into the underlying causes of this disorder. Here, we showed that bilateral infusion of 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) into the substantia nigra pars compacta (SNc) of Wistar rats caused degeneration of nigrostriatal dopaminergic neurons, cell loss in the hippocampal CA1 area, as well as microglial activation and increase of interleukin-2 levels in several brain regions. In addition, increase of anxiety-like behavior and impairment of object recognition were observed in the MPTP-lesioned rats. These findings suggest that neuroinflammation may contribute to MPTP-induced neurodegeneration and behavioral deficits, which is suggested as an animal model of Parkinson's disease dementia.

Keywords: cytokines, dementia, object recognition, neuroinflammation, microglial activation

Parkinson's disease (PD) is a neurodegenerative disorder with a high prevalence (Aarsland, Zaccai, & Brayne, 2005). In addition to the motor dysfunctions, cognitive impairment and dementia are seen in a high percentage of patients with PD during the late course and even at the beginning of the disease (Brown & Marsden, 1984; Owen et al., 1995). The proportion of patients with PD with dementia is around 25% to 30%, an incidence up to six times higher than that in healthy people (Aarsland et al., 2001). Emotional changes (Levin et al., 1991), psychotic symptoms (Emre, 2003), working memory, as well as visuospatial dysfunctions (Crucian & Okun, 2003; Emre, 2003) are the main symptoms in Parkinson's disease dementia (PDD).

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Physiologically, PD is mainly characterized by the loss of dopamine (DA)-containing neurons in the substantia nigra (McGeer & McGeer, 2004), and inflammation has been proposed as a possible mechanism in the pathogenesis of PD (Abramsky & Litvin, 1978; McGeer & McGeer, 2004). 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, Itagaki, Boyes, & McGeer, 1988; McGeer & McGeer, 1995; Sawada, Imamura, & Nagatsu, 2006), which has been suggested to be responsible for neuronal dysfunction and cognitive decline in PD (Imamura et al., 2005). Activated microglia produces a variety of inflammatory cytokines, including interleukin (IL)-2 (Allan & Rothwell, 2001). Increased levels of inflammatory cytokines have also been found in the nigrostriatal regions and in cerebrospinal fluid (CSF) of patients with PD (Mogi, Harada, Kondo, et al., 1994; Mogi, Harada, Riederer, et al., 1994; Mogi et al., 1998; Nagatsu, Mogi, Ichinose, & Togari, 2000a, 2000b; Nagatsu & Sawada, 2005, 2006). Because DAergic neurons appear to be particularly vulnerable to inflammatory cytokines (McGeer & McGeer, 2004), these cytokines have been implicated in cognitive impairment in PD (Kim & Joh, 2006; Langford & Masliah, 2001). Clinical studies have shown that IL-2 levels are increased in the caudate nucleus (Mogi, Harada, Kondo, Riederer, & Nagatsu, 1996) and in the CSF (Mogi, Harada, Narabayashi et al., 1996) in patients with PD, which are correlated to psychiatric disorders (Licinio, Seibyl, Altemus, Charney, & Krystal, 1993; McAllister et al., 1995). In addition, IL-2 immunotherapy of cancer patients is associated with pronounced cognitive disturbances (Caraceni et al., 1993; Denicoff et al., 1987; West et al., 1987), particularly in tests involving spatial learning and memory (Caraceni et al., 1993).

In spite of current knowledge about cognitive impairment in PDD, research has been hampered by the lack of a suitable animal

model of dementia in this disease. Thus, it is necessary to establish an animal model of PDD in which behavioral changes occur after degeneration of the nigrostriatal DAergic system. Animals treated with 1-methyl-4-phenyl-1,2,3,6-tetrahydropyriding (MPTP) are widely used as a PD animal model because, after converting to 1-methyl-4-phenylpyridinium (MPP^+), MPTP selectively damages the DAergic system and causes microglial activation coupled to increased levels of inflammatory cytokines, including IL-2, in humans (Mogi, Harada, Narabayashi, et al., 1996). Impairment of executive and visuospatial functions is observed not only in patients with PDD (Crucian & Okun, 2003; Emre, 2003), but also in people exposed to MPTP (Stern, Tetrud, Martin, Kutner, & Langston, 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. Accordingly, with the objective of establishing a rat model of PDD, we examined motor and emotional behavior as well as object recognition in rats after MPTP lesion using a battery of behavioral tests. In addition, we analyzed microglial activation and IL-2 levels in the brain. Our results suggest that neuroinflammation may contribute to MPTP-induced neurodegeneration and behavioral deficits, which can be a possible animal model of PDD.

Method

Subjects

Male Wistar rats (415.9 ± 4.0 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-hr light–dark cycle (lights on at 07:00 h) with food and water available ad libitum. Each rat was handled and gentled for 5 min/day on 3 consecutive days, starting one day after arrival. All experimental procedures were performed according to the NIH Guide for the Care and Use of Laboratory Animals and were approved by the Animal Care Committee of Chung Shan Medical University (IACUC approval No.: 434).

General Procedure

All rats 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, MO, USA; Da Cunha et al., 2001; Gevaerd, Takahashi, Silveira, & Da Cunha, 2001) or vehicle (Day 0) (see Surgery section below). They were then subjected to a bar test on Days 3, 5, 7, and 9 and to an elevated plus-maze test on Day 11, followed by an object recognition test starting on Day 11, and finishing on Day 13, all of which were started at least 2 h after the beginning of the light-phase (7:00 h). For behavioral testing, the rats were weighed in the animal room, placed individually in a clean cage (25 × 41 × 19 cm), and transported to a dim observation room (28 lux red light). The test equipment was cleaned using 20% ethanol and thoroughly dried before each test trial.

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

On Day 14 after MPTP lesion, the rats were sacrificed by exposure to CO_2, transcardially perfused with phosphate-buffered saline (PBS), and the brain was immediately removed to measure IL-2 levels. For histochemical assessment, 4 to 6 randomly selected rats per group were further perfused with 4% paraformaldehyde in PBS and the brain was removed and postfixed in 20% sucrose solution with 4% paraformaldehyde at 4° C.

Surgery

Brain surgery was performed on a stereotaxic instrument. The rats received intraperitonial injection (IP) of atropine sulfate (0.4 mg/kg, IP) to suppress salivation and were anesthetized using Zoletil (2 mg/kg, IP; Virbac, Carros, France). In the treatment 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 & Watson, 1986): anterio-posterial: −5.0 mm, middle-leteral: ± 2.0 mm, ventral depth: −8.0 mm from the bregma, midline, and skull surface, respectively. Controls were subjected to the same procedure, but were infused with 2 μl of saline instead of MPTP ($n = 15$). Immediately after surgery, the rats were intramuscular injected (IM) with penicillin-G procaine (0.2 ml, 20,000 IU, IM) and housed individually in plastic cages (25 × 41 × 19 cm) for 10 days, then they were regrouped in their home cages (rats from the same home cage underwent the same treatment). During the first 5 postoperative 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).

Behavioral Tests

Bar test. The bar test is the most commonly used method to determine the intensity of catalepsy. In this test, the rat is placed in an unusual posture and the time it takes to correct its posture to normal is recorded (Sanberg, Bunsey, Giordano, & Norman, 1988). The bar test was performed on Days 3, 5, 7, and 9 after surgery. 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. Each rat was tested in 3 consecutive trials on each testing day.

Elevated plus-maze test. Unconditioned anxiety-like avoidance behavior was assessed using the elevated plus-maze test, performed on Day 11. The construction of the elevated plus-maze and the testing procedures were the same as in our previous report (Ho et al., 2005). 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) risk assessment, that is, the rat showed head dipping but its body was still in the enclosed arm during this behavior, and (5) total distance, that is, the distance traveled by the rat in cm. Entry into any compartment was defined as the center of the rat's body entering the compartment.

Object recognition test. The apparatus and testing procedure for the object recognition test were similar to those described

previously (Mumby, Tremblay, Lecluse, & Lehmann, 2005). An open field arena was constructed of black polyvinyl plastic (100 cm long × 100 cm wide × 60 cm high) (Figure 2A). Each rat was subjected to 3 exposure sessions at 24 h intervals. Five minutes after the last exposure session, a test trial was performed. After placing the rat in the open field, the experimenter left the room to avoid interaction with the rat during testing. 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 11 (5 min after the elevated plus-maze test), each rat was habituated to the open field individually. The rat was placed in the only free corner and allowed to explore the objects for 5 min each on 3 consecutive days. Five minutes after the last exposure session, object "B" was replaced by a novel object "D" and the rat was returned from its home cage 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 was recorded. The percentage of the exploration time spent on object B or D in the session [($\text{Time}_{\text{B or D}}/\text{Time}_{\text{all objects}}$) × 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. The objects were thoroughly cleaned with 20% alcohol before use for each rat.

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) or rat MHC class II (OX-6; 1:400; BD Biosciences Pharmingen, CA) diluted in PBS. OX-6 selectively stains mainly activated microglia (Ogura, Ogawa, & Yoshida, 1994). The sections were incubated sequentially for 30 min at 37 °C with biotinylated horse antimouse IgG antibody (Vector Laboratory, CA) and avidin-biotin-horseradish peroxidase complex (ABC Elite HRP kit; Vector Laboratory), then were incubated for 30 min at room temperature with 0.02% 3,3′-diaminobenzidine (Sigma). The reaction was stopped by extensive washing with PBS. In sections containing hippocampus, Nissl staining was used to identify neurons.

Image analysis. The stained brain sections, identified according to the rat brain atlas (Paxinos & 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) and the Image Pro Plus Software 4.1 (Media Cybernetics, CA). In this study, three squares of area of interest were created, measuring 61,104, 23,409, and 142,129 μm^2 to determine the optical density of TH immunoreactivity in the striatum and neuronal density in the SNc and hippocampal CA1 area, respectively. 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 nonimmunoreactive 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. The density of activated microglia was measured, as the methods described in the literature (Sugama et al., 2003), in single sections placed at equidistant positions in the brain areas. The number of activated microglial cells in the SNc, amygdala, and hippocampus was counted in the areas of interest, measuring 85,758, 196,588, and 458,615 μm^2, respectively. Because the neurons are tightly packed, it is difficult to directly calculate the number of pyramidal neurons in the CA1 area from a 30 μm-thick brain section. Thus, a semiquantitative method, percentage of area of Nissl-stained neurons in an area of interest in the CA1 area, was used to represent the neuronal density, where the neurons in the CA1 area were always included in the diagonal of the area of interest.

Measurement of IL-2 Levels

The prefrontal cortex (the rostral part of the cortex, not including the forceps minor of the corpus callosum, about 12 mm anterior of the coronal plane passing through the interaural line, according to the rat brain atlas; Paxinos & Watson, 1986), nonprefrontal cortex, amygdala, striatum (ventral and dorsal part), and hippocampus were dissected on an ice-cold plate and stored at −80 °C until use. The procedures for IL-2 measurement were identical to those used in our previous report (Ho et al., 2007). Briefly, IL-2 levels in a sample containing about 30–40 μg of total protein were measured using an enzyme linked immunosorbent assay (ELISA) kit with monoclonal antirat IL-2 antibody (CytoSets, BioSourse, CA) according to the manufacturer's instructions.

Data Analysis

Statistical testing was performed to compare two groups using the t test. Analysis of variance (ANOVA) for repeated measures, followed by Scheffé's post hoc test, and evaluation of correlations, Pearson's correlation coefficient, between variables were used when appropriate. All results are expressed as the mean ± *SEM*. The level of significance was defined as $p < .05$.

Results

Behavior

After MPTP lesion, the rats showed a significant and transitory catalepsy, that is, an increase in the crossing latency in the bar test. ANOVA with repeated measures revealed significant main effects of time, $F(3, 114) = 10.46$, $p < .001$ and treatment, $F(1, 38) = 7.97$, $p < .01$, but no significant time × treatment interaction. As shown in Figure 1, significant longer crossing latencies in the MPTP-lesioned rats, indicating catalepsy, were seen on Days 3, 5, and 7 after MPTP lesion ($df = 38$, $t \geqq 2.21$, $ps < .05$, 1-tailed), while, on Day 9, MPTP- and vehicle-treated rats displayed comparable crossing latencies.

The longer open arm latency, lower open arm time, and less number of risk assessment in the elevated plus-maze test were seen in MPTP-treated rats on Day 11 ($df = 38$, t values >2.13, $ps < .05$), indicating increased anxiety-like levels. However, the

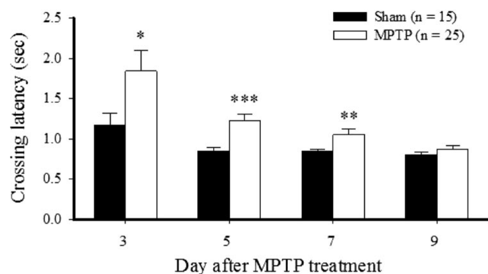

Figure 1. Effects of 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) on catalepsy in the bar test. MPTP was bilaterally infused into the substantia nigra pars compacta, then the catalepsy bar test was performed on Days 3, 5, 7, and 9 after the lesion. $^{*}p < .05$, $^{**}p < .01$, $^{***}p < .001$ compared with the sham-operated group on the given day. The data are expressed as the mean ± *SEM* for the indicated number of rats.

number of enclosed arm entries and total distance traveled in the elevated plus-maze test, which is commonly used to measure general activity, were not different between the groups (see Table 1).

MPTP-treated rats spent a lower percentage of time exploring the novel object "D" on Day 13 after lesion than the vehicle-treated controls ($df = 38$, $t = 2.24$, $p < .05$) (Figure 2B). As shown in Figure 2C, ANOVAs with repeated measures revealed a significant time effect, $F(3, 114) = 26.67$, $p < .001$ for the rearing number, but no significant treatment effect or time × treatment interaction.

IL-2 Levels

On Day 14 after the MPTP lesion, IL-2 levels were significantly increased in the striatum, amygdala, and nonprefrontal cortex, compared with the vehicle-treated group ($df = 28$, $t \geqq 4.97$, $ps < .001$), but were unchanged in the prefrontal cortex and hippocampus (see Figure 3). IL-2 levels in the prefrontal cortex were negatively correlated with open arm latency and enclosed arm time ($ps < .01$) but positively correlated with open arm time and open arm entry in the elevated plus-maze test ($ps < .05$). Further, risk assessment was negatively correlated with the levels of IL-2 in the striatum and amygdala ($ps < .05$); open arm latency was positively correlated with IL-2 levels in the nonprefrontal cortex ($p < .05$; Table 2).

Histology

Representative photomicrographs of immunostained and Nissl-stained brain sections are shown in Figures 4–6. TH positive neurons were found in the striatum and SNc of the brain from sham-operated and MPTP-lesioned groups. TH immunoreactivity was observed in neuronal cell bodies and their processes. The resolution of the TH staining was sufficient for counting the cell number in an area of interest under light microscopy. MPTP lesion induced a reduction in relative optical density of TH immunoreactivity in the striatum ($df = 10$, $t = 3.75$, $p < .01$) and a decrease in density of DAergic neurons in the SNc ($df = 10$, $t = 4.17$, $p < .01$), compared with the sham-operated group (see Figure 4). Microglial activation, indicated by an accumulation of OX-6-positive cells, was evident in the SNc, amygdala, and hippocampus of MPTP-lesioned rats, where the densities of activated microglia were 1361 ± 531, 1039 ± 188, and 1145 ± 206, per mm^2, respectively ($n = 4$ for each group). However, no activated microglia was observed in sham-operated rats (see Figure 5). Semi-quantitative analysis confirmed that MPTP lesion decreased the neuronal density in the pyramidal cell layer in the hippocampal CA1 area ($df = 8$, $t = 4.17$, $p < .01$), compared with the sham-operated rats (see Figure 6).

Discussion

Intra-SNc infusion of MPTP caused degeneration of the nigrostriatal DAergic system, cell loss in the hippocampal CA1 area, microglial activation in the SNc, amygdala, and hippocampus, and an increase of IL-2 levels in the striatum, amygdala, and nonprefrontal cortex. Furthermore, emotion-related and cognitive changes were also observed after MPTP lesion, namely, increased anxiety-like avoidance behavior and impairment of object recognition. We did not find evidence of motor impairment 9 days after MPTP lesion when catalepsy was no longer observed, suggesting that the differences seen in subsequent tests were not attributable to motor deterioration. These results suggest that neuroinflammation might be responsible for cognitive decline in the early phase of DAergic degeneration.

PD is characterized by specific degeneration of DAergic neurons in the substantia nigra and the resulting loss of the nerve terminals in the striatum. Consistent with report showing that neurophathological features in an experimental MPTP PD model are very similar to that found in PD itself (Kim & Joh, 2006), the current study indicated that intra-SNc infusion of MPTP resulted in a reduction of TH immunoreactivity in the striatum and a decrease in neuronal density in the SNc, indicating DAergic lesions. Although, in the present study, calculating the cell number in the representative brain sections yielded similar histological results with previous reports, stereological approach by counting cells in a complete series of sections can provide additional data (Ferro et al., 2005; Meissner et al., 2003). In line with previous reports (Ferro et al., 2005; Sedelis, Schwarting, & Huston, 2001), transitory catalepsy was demonstrated using the bar test. This catalepsy was seen during the first 7 days, but not on Day 9 after MPTP lesion. Recovery of motor function was further supported by the lack of differences between the groups in the number of enclosed arm entries and distance traveled in the elevated plus-

Table 1
Effects of 1-Methyl-4-Phenyl-1,2,3,6-Tetrahydropyridine (MPTP) on Behavior in the Elevated Plus-Maze Test on Day 11

	Sham (n = 15)	MPTP (n = 25)
Open arm latency (s)	94.2 ± 25.2	179.3 ± 28.9*
Open arm time (s)	50.7 ± 10.8	24.2 ± 7.2*
Enclosed arm time (s)	191.3 ± 16.3	244.7 ± 9.0**
Open arm entry (no.)	8.0 ± 1.6	2.5 ± 0.6**
Enclosed arm entry (no.)	9.0 ± 0.8	7.0 ± 0.9
Risk assessment (no.)	3.5 ± 0.4	1.9 ± 0.3**
Total distance (cm)	2621.0 ± 323.0	2142.6 ± 241.1

Note. The data are expressed as the mean ± *SEM* for the indicated number of animals.
$^{*}p < .05$. $^{**}p < .01$, compared with the sham-operated group.

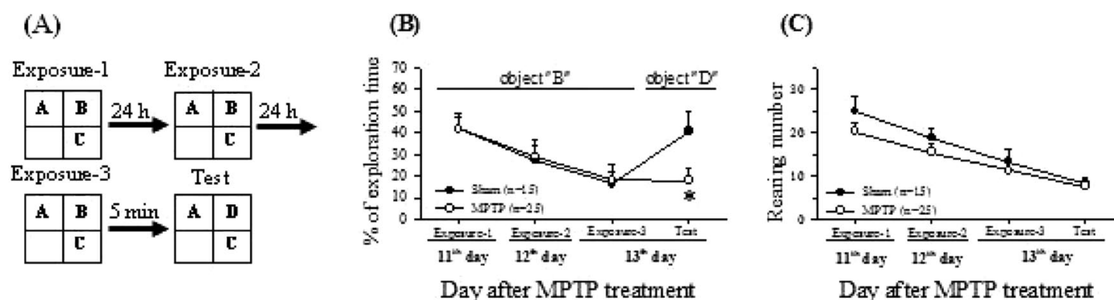

Figure 2. Effects of 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) on behavior in the open field object recognition test. (A) shows a schematic drawing of the arrangement of the objects in the test. Rats received 3 exposure sessions (5 min each) at 24-h intervals, then were tested 5 min after Session 3. In the test trial, object "B" was replaced by a novel object "D." This test was performed on Days 11–13 after MPTP lesion. (B) percentage of time spent exploring object "B" or "D." (C) rearing number appeared in the exposure and test trials. The data are expressed as the mean ± *SEM* for the indicated number of rats. * $p < .05$ compared with the sham-operated group at the given time point.

maze test and in the rearing number in the object recognition test, which indicated absence of gross motor impairment, suggesting that the behavioral performance in the tests was not confounded by motor impairment or general sickness. Striatal reinnervation following lesions of SNc DAergic neurons provides a possible compensatory mechanism of motor recovery in rats (Stanic, Finkelstein, Bourke, Drago, & Horne, 2003). Although no motor recovery has been seen in the progression of PD in humans, the ability of MPTP-treated rats to recover motor function makes it possible to study behavioral changes in these rats as a model for the motor presymptomatic phase of PD.

Hippocampal dysfunction may be involved in visuospatial deficits observed in patients with PD (Girotti et al., 1988). It has been reported that MPTP-treated rats show deficits in performing a spatial working memory in the Morris water-maze task (Da Cunha et al., 2006; Ferro et al., 2005) and in acquisition and retention processes in the active avoidance test (Da Cunha et al., 2001), suggesting that MPTP-treated rats may be a model for early PD amnesia (Perry et al., 2005). The hippocampus is important for spatial navigation (Zhang, Pothuizen, Feldon, & Rawlins, 2004), recognition memory (Broadbent, Squire, & Clark, 2004), working memory (Ohno, Kobayashi, Kishi, & Watanabe, 1997; Ohno & Watanabe, 1996; Schuster & Schmidt, 1992), as well as short-term memory associating objects and their locations (Piekema, Kessels, Mars, Petersson, & Fernandez, 2006). Moreover, the hippocampal CA1 region plays a critical role in object recognition (Wood, Mumby, Pinel, & Phillips, 1993). Ischemia-induced cell loss in the hippocampal CA1 area of rats resulted in object recognition deficits in the acquisition and retention in delayed nonmatching-to-sample task (Wood et al., 1993). Further, local pharmacological manipulations in the CA1 area also affected consolidation of object recognition memory (Clarke et al., 2008; de Lima, Luft, Roesler, & Schroder, 2006). In agreement with the above literatures, our present data show that MPTP lesion causes neuroinflammation and cell loss in the hippocampal CA1 pyramidal neurons, providing a plausible mechanism underling the object recognition impairments, that is, a reduced time spent exploring a novel object, seen in MPTP-treated rats. Dysfunction of object recognition in animal model may be compatible with some phenomenon of agnosia in demented patients. It has been reported that cognitively deteriorated patients with PD performed more poorly than the healthy controls and cognitively preserved patients with PD 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, Revonsuo, Pihko, Portin, & Rinne, 2004). It is noteworthy that the rats in the current study may not totally rely on visual sensation to recognize the objects. In addition, striatal dysfunction because of DAergic degeneration may have also contributed to the object-approach deficits because the functional units in the striatum integrate the sensory information and movements related to them (Da Cunha et al., 2009). Hence, the MPTP-lesioned rats may not able to encode objects and the action of approaching them. Still, the present data may prove to be a useful tool in assessing the ability of pharmacological agents to prevent symptoms in neurodegeneration-related cognitive deficits.

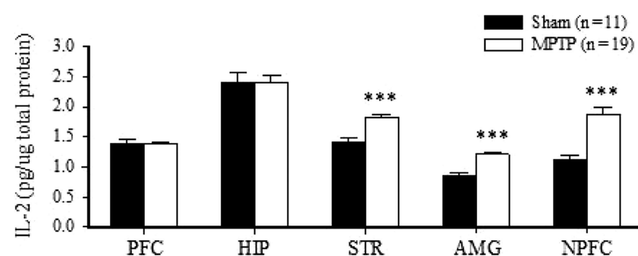

Figure 3. Effects of 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) on interleukin (IL)-2 levels in the brain. The brain tissue was taken 14 days after MPTP lesion. A significant increase in IL-2 levels is seen in the striatum (STR), amygdala (AMG), and nonprefrontal cortex (NPFC), but not the prefrontal cortex (PFC) and hippocampus (HIP). The data are expressed as the mean ± *SEM* for the indicated number of rats. *** $p < .001$ compared with the sham-operated group, *t* test.

Microglia, the resident immune cells of the central nervous system, act as regulators of the secretion of neurotrophic and neurotoxic factors, for example, cytokines (Kadiu, Glanzer, Kipnis, Gendelman, & Thomas, 2005). Microglial activation and increased levels of inflammatory cytokines have been observed in

Table 2
Correlations Between Interleukin (IL)-2 Levels in the Brain and Behavior in the Elevated Plus-Maze Test

	IL-2 level									
	Prefrontal cortex		Hippocampus		Striatum		Amygdala		Nonprefrontal cortex	
	Pearson correlation	*p*	Pearson correlation	*p*	Pearson correlation	*p*	Pearson correlation	*p*	Pearson correlation	*p*
Open arm latency (s)	−0.516	.003**	−0.036	.853	0.347	.060	0.124	.514	0.455	.012*
Open arm time (s)	0.562	.001***	0.29	.127	−0.307	.099	−0.078	.681	−0.268	.152
Enclosed arm time (s)	−0.555	.001***	0.001	.996	0.357	.053	0.168	.376	0.298	.110
Open arm entry (no.)	0.429	.018*	0.276	.148	−0.324	.080	−0.072	.706	−0.205	.278
Enclosed arm entry (no.)	0.128	.502	0.266	.163	−0.166	.380	0.195	.301	0.04	.836
Risk assessment (no.)	0.293	.117	0.088	.650	−0.396	.030*	−0.387	.035*	−0.292	.118
Total distance (cm)	0.424	.055	0.212	.369	−0.18	.434	0.24	.296	−0.347	.123

Note. *p* values are the significance calculated by 2-tailed.

the substantia nigra, putamen, and hippocampus in patients with PD (McGeer et al., 1988; McGeer & McGeer, 1995; Sawada et al., 2006). Activated microglia at the site of inflammation change their morphology, become phagocytic, and release proinflammatory cytokines as well as neurotoxins (Dheen, Kaur, & Ling, 2007; Imamura et al., 2003; Teismann & Schulz, 2004), which may amplify inflammatory response, cause neuronal damage, and have been implicated in pathogenesis of PD (Kim & Joh, 2006). Furthermore, inflammation has been suggested to be responsible for neuronal degeneration and cognitive decline in PD and dementia with Lewy bodies (Imamura et al., 2005). Similarly, the present study shows that MPTP lesion causes cell loss in the hippocampal CA1 area, microglial activation in the SNc, amygdala, and hippocampus, as well as increased levels of IL-2 in the striatum, amygdala, and nonprefrontal cortex. Although the loss of DAergic neurons and terminals was observed in the SNc and striatum, respectively, activated microglia was found in the SNc but not the striatum, maybe because the glial response is more robust in the SNc than in the

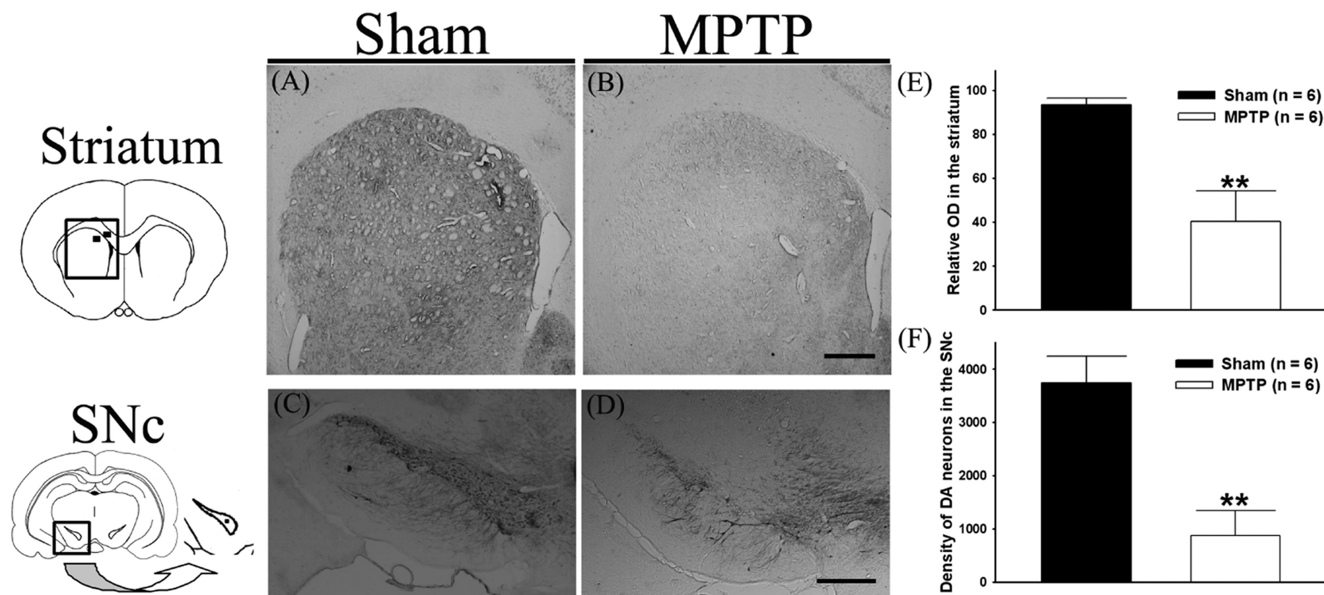

Figure 4. Effects of 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) on dopamine (DA)-containing neurons in the striatum and substantia nigra pars compacta (SNc). DAergic neurons, stained by tyrosine hydroxylase (TH) immunoreaction, are shown in the representative coronal sections of the striatum (A, B) and SNc (C, D) (magnification, × 50; bar, 500 μm) in sham-operated and MPTP-treated rats. Black squares 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-lesioned rats show decreases in relative OD of TH immunoreactivity in the striatum (E) and in density of DAergic neurons in the SNc (F). ** $p < .01$ compared with corresponding sham-operated rats. The schematic drawings are modified from the rat brain atlas (Paxinos & Watson, 1986). Adapted with permission.

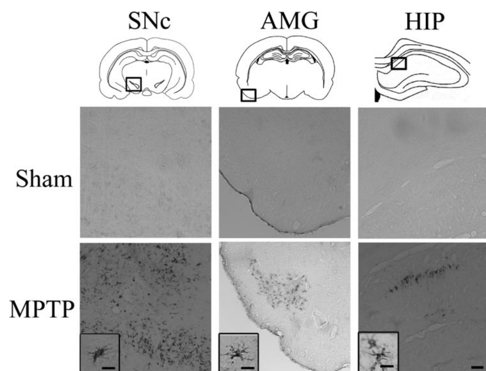

Figure 5. Effects of 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) on microglial activation in the brain. No activated microglia, OX-6-positive cells, was found in the substantia nigra pars compacta (SNc), amygdala (AMG), and hippocampus (HIP) of sham-operated rats. MPTP lesion caused a massive accumulation of activated microglia in the SNc, AMG, and HIP (magnification, × 100; bar, 100 μm). Insets show activated microglia at magnification of × 400, bar, 20 μm. Adapted with permission.

striatum when DAergic degeneration occurs (McGeer et al., 1988) and/or that transient activation of microglia in the striatum had already disappeared before the brain was taken (Kohutnicka, Lewandowska, Kurkowska-Jastrzebska, Czlonkowski, & Czlonkowska, 1998). Moreover, microglia are thought to be a principal source of cytokines (Kim & Joh, 2006), but other mechanisms underling IL-2 production, for example astrocytes and tissue infiltrating immune cells (Kohutnicka et al., 1998), should be considered as the regions of microglial activation were not related to the place showing IL-2 elevations.

A variety of cytokines, for example tumor necrosis factor-alpha (TNF-α) and IL-1β, has been shown to be increased in the SNc of patients with PD (Mogi, Harada, Riederer, et al., 1994) and to colocalize with glia cells (Boka et al., 1994; Hunot et al., 1999). MPTP is known to induce an elevation of cytokines, including IL-1, IL-2, and IL-6, in several brain regions (Mogi et al., 1998; Nagatsu et al., 2000b; Shen et al., 2005), but this has mostly been done in mice. The present data show that this is not species specific because IL-2 alteration was also observed in the brain of MPTP-treated rats. It has been known that IL-2 plays a role in cognitive function and in hippocampal neuronal development (Petitto, McNamara, Gendreau, Huang, & Jackson, 1999). IL-2 immunotherapy elicits cognitive impairment (Caraceni et al., 1993; Denicoff et al., 1987; West et al., 1987) and repeated injection of IL-2 induces abnormalities in novelty-induced locomotion, learning, and spatial memory in the Morris water-maze test, as well as altered exploratory activity in rodents (Lacosta, Merali, & Anisman, 1999; Zalcman, Murray, Dyck, Greenberg, & Nance, 1998; Zalcman, 2001). Therefore, increase of IL-2 in the brain may participate in MPTP-induced impairment of cognitive behavior.

Although DAergic deficit is the main neurochemical impairment in PD, clinical data show that dementia in PDD patients does not improve with levodopa treatment (Lewis, Slabosz, Robbins, Barker, & Owen, 2005). Further, motor symptoms and cognitive dysfunction in PDD patients are correlated strongly with non-DAergic systems (Levy et al., 2002). Clinical studies on patients with PD have shown that IL-2 levels are increased in the caudate nucleus (Mogi, Harada, Kondo, et al., 1996) and the CSF (Mogi, Harada, Narabayashi, et al., 1996). IL-2 levels in the CSF are related to psychiatric disorders (Licinio et al., 1993; McAllister et al., 1995). In the present study, MPTP lesion does not only cause increased anxiety-like behavior in an elevated plus-maze test but also a widespread increase in IL-2 levels in the striatum, amygdala, and nonprefrontal cortex. The literature and our previous study show that IL-2 levels were related to emotional behavior (Ho et al., 2007; Lee et al., 2008; Petitto, McCarthy, Rinker, Huang, & Getty, 1997). Parallel to the present results, we previously also showed that striatal IL-2 mRNA was negatively correlated with open arm time (Pawlak, Ho, Schwarting, & Bauhofer, 2003) and positively with prefrontal IL-2 mRNA (Pawlak, Schwarting, & Bauhofer, 2005). Even more, striatal IL-2 microinjections modulated anxiety-like behavior in the elevated plus-maze test (Pawlak & Schwarting, 2006), and induced avoidance behavior in an open field test (Karrenbauer et al., 2009). The present study showed that the IL-2 levels in the brain areas have positive and/or negative correlations with emotional behavior, suggesting that IL-2 in the brain may take part in MPTP-induced behavioral deficits and has site-specific effects.

Microglial activation is an indicator of neuroinflammation because it takes place if neuronal degeneration occurs and is accompanied by the release of several products that lead to cytotoxic

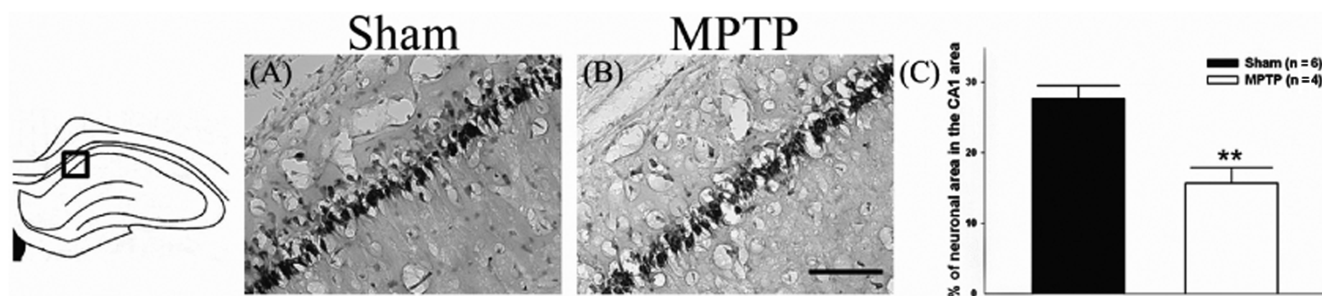

Figure 6. Effects of 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) on neurons in the hippocampal CA1 area. Images show Nissl-stained pyramidal neurons in the CA1 area of hippocampus, as indicated in the square of schematic drawing, in sham-operated (A) and MPTP-lesioned (B) rats (magnification, × 200; bar, 100 μm). MPTP-treated rats show decrease in neuronal area in the CA1 area. ** $p < .01$ compared with sham-operated rats (C). Adapted with permission.

and/or neurotrophic effects. The kinetic changes of neuroinflammation after MPTP lesion have been reported in the substantia nigra of mice, where transient microglial activation occurs from the Day 1 until Day 14 after MPTP lesion (Kohutnicka et al., 1998). Furthermore, an elevation of levels of a variety of cytokines was also observed in the CSF of MPTP-lesioned mice in the first 2 weeks (Yasuda et al., 2008). To avoid acute effects of stress on the brain, the rats were sacrificed one day after the last behavioral test. Thus, histological and IL-2 changes observed in this study should be a chronic state and are suggested to be associated with behavioral performance, notwithstanding the fact that other factors are probably also involved. Moreover, through neuroinflammation and releasing of cytokines, activated microglia might contribute to neurodegeneration in the nigrostriatal pathway (Sawada et al., 2006; Teismann & Schulz, 2004); inhibition of microglia activation prevents the loss of DAergic neurons in MPTP-lesioned rats (Wu et al., 2002). Besides, the involvement of cyclooxygenase Type II (COX-2), a key enzyme for the neuroinflammation process, in MPTP-induced neuronal and behavioral deficits should be considered because the expression of COX-2 and production of prostaglandin are up-regulated in DAergic neurons of patients with PD and MPTP-treated rodents (de Meira Santos Lima et al., 2006; Teismann et al., 2003). COX-2 inhibition has effects of behavioral and neuronal protection in MPTP-lesioned rats (Reksidler et al., 2007). Thus, suppression of neuroinflammation may therefore be an important 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, intranigral infusion of MPTP also caused increases in microglial activation and IL-2 levels, cell loss in the hippocampal CA1 area, as well as emotional and object recognition deficits. Thus, these results suggest that neuroinflammation might be involved in cognitive decline in the early phase of PD. The main advantage of this model is that it shows distinct behavioral impairment similar to those observed in PDD patients, but does not present motor deficits that would otherwise confound the interpretation of behavior (Ferro et al., 2005). The mechanisms by which activated microglia and IL-2 affect neuronal and behavioral function are not yet clear. If such a link could be established, clinical intervention trials with agents that dampen microglial activation might alleviate neurodegeneration/dementia and be warranted in PDD.

References

Aarsland, D., Andersen, K., Larsen, J. P., Lolk, A., Nielsen, H., & Kragh-Sorensen, P. (2001). Risk of dementia in Parkinson's disease: A community-based, prospective study. *Neurology, 56,* 730–736.

Aarsland, D., Zaccai, J., & Brayne, C. (2005). A systematic review of prevalence studies of dementia in Parkinson's disease. *Movement Disorders, 20,* 1255–1263.

Abramsky, O., & Litvin, Y. (1978). Autoimmune response to dopamine-receptor as a possible mechanism in the pathogenesis of Parkinson's disease and schizophrenia. *Perspectives in Biology and Medicine, 22,* 104–114.

Allan, S. M., & Rothwell, N. J. (2001). Cytokines and acute neurodegeneration. *Nature Reviews Neuroscience, 2,* 734–744.

Boka, G., Anglade, P., Wallach, D., Javoy-Agid, F., Agid, Y., & Hirsch, E. C. (1994). Immunocytochemical analysis of tumor necrosis factor and its receptors in Parkinson's disease. *Neuroscience Letters, 172,* 151–154.

Broadbent, N. J., Squire, L. R., & Clark, R. E. (2004). Spatial memory, recognition memory, and the hippocampus. *Proceedings of the National Academy of Science of the United States of America, 101,* 14515–14520.

Brown, R. G., & Marsden, C. D. (1984). How common is dementia in Parkinson's disease? *Lancet, 2,* 1262–1265.

Caraceni, A., Martini, C., Belli, F., Mascheroni, L., Rivoltini, L., Arienti, F., et al. (1993). Neuropsychological and neurophysiological assessment of the central effects of interleukin-2 administration. *European Journal of Cancer, 29*A, 1266–1269.

Clarke, J. R., Rossato, J. I., Monteiro, S., Bevilaqua, L. R., Izquierdo, I., & Cammarota, M. (2008). Posttraining activation of CB1 cannabinoid receptors in the CA1 region of the dorsal hippocampus impairs object recognition long-term memory. *Neurobiology of Learning and Memory, 90,* 374–381.

Crucian, G. P., & Okun, M. S. (2003). Visual-spatial ability in Parkinson's disease. *Frontiers in Bioscience, 8,* S992–S997.

Da Cunha, C., Gevaerd, M. S., Vital, M. A., Miyoshi, E., Andreatini, R., Silveira, R., et al. (2001). Memory disruption in rats with nigral lesions induced by MPTP: A model for early Parkinson's disease amnesia. *Behavioural Brain Research, 124,* 9–18.

Da Cunha, C., Silva, M. H., Wietzikoski, S., Wietzikoski, E. C., Ferro, M. M., Kouzmine, I., et al. (2006). Place learning strategy of substantia nigra pars compacta-lesioned rats. *Behavioral Neuroscience, 120,* 1279–1284.

Da Cunha, C., Wietzikoski, E. C., Dombrowski, P., Bortolanza, M., Santos, L. M., Boschen, S. L., et al. (2009). Learning processing in the basal ganglia: A mosaic of broken mirrors. *Behavioural Brain Research, 199,* 157–170.

de Lima, M. N., Luft, T., Roesler, R., & Schroder, N. (2006). Temporary inactivation reveals an essential role of the dorsal hippocampus in consolidation of object recognition memory. *Neuroscience Letters, 405,* 142–146.

de Meira Santos Lima, M., Braga Reksidler, A., Marques Zanata, S., Bueno Machado, H., Tufik, S., & Vital, M. A. (2006). Different parkinsonism models produce a time-dependent induction of COX-2 in the substantia nigra of rats. *Brain Research, 1101,* 117–125.

Denicoff, K. D., Rubinow, D. R., Papa, M. Z., Simpson, C., Seipp, C. A., Lotze, M. T., et al. (1987). The neuropsychiatric effects of treatment with interleukin-2 and lymphokine-activated killer cells. *Annals of Internal Medicine, 107,* 293–300.

Dheen, S. T., Kaur, C., & Ling, E. A. (2007). Microglial activation and its implications in the brain diseases. *Current Medicinal Chemistry, 14,* 1189–1197.

Emre, M. (2003). Dementia associated with Parkinson's disease. *Lancet Neurol, 2,* 229–237.

Ferro, M. M., Bellissimo, M. I., Anselmo-Franci, J. A., Angellucci, M. E., Canteras, N. S., & Da Cunha, C. (2005). 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. *Journal of Neuroscience Methods, 148,* 78–87.

Gevaerd, M. S., Takahashi, R. N., Silveira, R., & Da Cunha, C. (2001). Caffeine reverses the memory disruption induced by intra-nigral MPTP-injection in rats. *Brain Research Bulletin, 55,* 101–106.

Girotti, F., Soliveri, P., Carella, F., Piccolo, I., Caffarra, P., Musicco, M., et al. (1988). Dementia and cognitive impairment in Parkinson's disease. *Journal of Neurology, Neurosurgery and Psychiatry, 51,* 1498–1502.

Ho, Y. J., Hsu, L. S., Wang, C. F., Hsu, W. Y., Lai, T. J., Hsu, C. C., et al. (2005). Behavioral effects of D-cycloserine in rats: The role of anxiety level. *Brain Research, 1043,* 179–185.

Ho, Y. J., Wang, C. F., Hsu, W. Y., Tseng, T., Hsu, C. C., Kao, M. D., et al. (2007). Psychoimmunological effects of dioscorea in ovariectomized rats: Role of anxiety level. *Annals of General Psychiatry, 6,* 21.

Hunot, S., Dugas, N., Faucheux, B., Hartmann, A., Tardieu, M., Debre, P., et al. (1999). FcepsilonRII/CD23 is expressed in Parkinson's disease and

induces, in vitro, production of nitric oxide and tumor necrosis factor-alpha in glial cells. *Journal of Neuroscience, 19,* 3440–3447.

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

Imamura, K., Hishikawa, N., Sawada, M., Nagatsu, T., Yoshida, M., & Hashizume, Y. (2003). Distribution of major histocompatibility complex class II-positive microglia and cytokine profile of Parkinson's disease brains. *Acta Neuropathology, 106,* 518–526.

Kadiu, I., Glanzer, J. G., Kipnis, J., Gendelman, H. E., & Thomas, M. P. (2005). Mononuclear phagocytes in the pathogenesis of neurodegenerative diseases. *Neurotoxicity Research, 8,* 25–50.

Karrenbauer, B. D., Ho, Y. J., Ludwig, V., Löohn, J., Spanagel, R., Schwarting, R. K., et al. (2009). Time-dependent effects of striatal interleukin-2 on open field behaviour in rats. *Journal of Neuroimmunology, 208,* 10–18.

Kim, Y. S., & Joh, T. H. (2006). Microglia, major player in the brain inflammation: Their roles in the pathogenesis of Parkinson's disease. *Experimental and Molecular Medicine, 38,* 333–347.

Kohutnicka, M., Lewandowska, E., Kurkowska-Jastrzebska, I., Czlonkowski, A., & Czlonkowska, A. (1998). Microglial and astrocytic involvement in a murine model of Parkinson's disease induced by 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP). *Immunopharmacology, 39,* 167–180.

Laatu, S., Revonsuo, A., Pihko, L., Portin, R., & Rinne, J. O. (2004). Visual object recognition deficits in early Parkinson's disease. *Parkinsonism and Related Disorders, 10,* 227–233.

Lacosta, S., Merali, Z., & Anisman, H. (1999). Influence of acute and repeated interleukin-2 administration on spatial learning, locomotor activity, exploratory behaviors, and anxiety. *Behavioral Neuroscience, 113,* 1030–1041.

Langford, D., & Masliah, E. (2001). Crosstalk between components of the blood brain barrier and cells of the CNS in microglial activation in AIDS. *Brain Pathology, 11,* 306–312.

Lee, Y. T., Wang, W. F., Cheng, C. W., Wu, S. L., Pawlak, C. R., & Ho, Y. J. (2008). Effects of escapable and inescapable stressors on behavior and interleukin-2 in the brain. *Neuroreport, 19,* 1243–1247.

Levin, B. E., Llabre, M. M., Reisman, S., Weiner, W. J., Sanchez-Ramos, J., Singer, C., et al. (1991). Visuospatial impairment in Parkinson's disease. *Neurology, 41,* 365–369.

Levy, G., Tang, M. X., Louis, E. D., Cote, L. J., Alfaro, B., Mejia, H., et al. (2002). The association of incident dementia with mortality in PD. *Neurology, 59,* 1708–1713.

Lewis, S. J., Slabosz, A., Robbins, T. W., Barker, R. A., & Owen, A. M. (2005). Dopaminergic basis for deficits in working memory but not attentional set-shifting in Parkinson's disease. *Neuropsychologia, 43,* 823–832.

Li, J. S., & Chao, Y. S. (2008). Electrolytic lesions of dorsal CA3 impair episodic-like memory in rats. *Neurobiology of Learning and Memory, 89,* 192–198.

Licinio, J., Seibyl, J. P., Altemus, M., Charney, D. S., & Krystal, J. H. (1993). Elevated CSF levels of interleukin-2 in neuroleptic-free schizophrenic patients. *The American Journal of Psychiatry, 150,* 1408–1410.

McAllister, C. G., van Kammen, D. P., Rehn, T. J., Miller, A. L., Gurklis, J., Kelley, M. E., et al. (1995). Increases in CSF levels of interleukin-2 in schizophrenia: Effects of recurrence of psychosis and medication status. *The American Journal of Psychiatry, 152,* 1291–1297.

McGeer, P. L., Itagaki, S., Boyes, B. E., & McGeer, E. G. (1988). Reactive microglia are positive for HLA-DR in the substantia nigra of Parkinson's and Alzheimer's disease brains. *Neurology, 38,* 1285–1291.

McGeer, P. L., & McGeer, E. G. (1995). The inflammatory response system of brain: Implications for therapy of Alzheimer and other neurodegenerative diseases. *Brain Research Reviews, 21,* 195–218.

McGeer, P. L., & McGeer, E. G. (2004). Inflammation and neurodegeneration in Parkinson's disease. *Parkinsonism and Related Disorders, 10*(Suppl. 1), S3–7.

Meissner, W., Prunier, C., Guilloteau, D., Chalon, S., Gross, C. E., & Bezard, E. (2003). Time-course of nigrostriatal degeneration in a progressive MPTP-lesioned macaque model of Parkinson's disease. *Molecular Neurobiology, 28,* 209–218.

Mogi, M., Harada, M., Kondo, T., Riederer, P., Inagaki, H., Minami, M., et al. (1994). Interleukin-1 beta, interleukin-6, epidermal growth factor and transforming growth factor-alpha are elevated in the brain from parkinsonian patients. *Neuroscience Letters, 180,* 147–150.

Mogi, M., Harada, M., Kondo, T., Riederer, P., & Nagatsu, T. (1996). Interleukin-2 but not basic fibroblast growth factor is elevated in parkinsonian brain. Short communication. *Journal of Neural Transmission, 103,* 1077–1081.

Mogi, M., Harada, M., Narabayashi, H., Inagaki, H., Minami, M., & Nagatsu, T. (1996). 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. *Neuroscience Letters, 211,* 13–16.

Mogi, M., Harada, M., Riederer, P., Narabayashi, H., Fujita, K., & Nagatsu, T. (1994). Tumor necrosis factor-alpha (TNF-alpha) increases both in the brain and in the cerebrospinal fluid from parkinsonian patients. *Neuroscience Letters, 165,* 208–210.

Mogi, M., Togari, A., Ogawa, M., Ikeguchi, K., Shizuma, N., Fan, D., et al. (1998). Effects of repeated systemic administration of 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) to mice on interleukin-1beta and nerve growth factor in the striatum. *Neuroscience Letters, 250,* 25–28.

Mumby, D. G., Tremblay, A., Lecluse, V., & Lehmann, H. (2005). Hippocampal damage and anterograde object-recognition in rats after long retention intervals. *Hippocampus, 15,* 1050–1056.

Nagatsu, T., Mogi, M., Ichinose, H., & Togari, A. (2000a). Changes in cytokines and neurotrophins in Parkinson's disease. *Journal of Neural Transmission, Supplementum,* 277–290.

Nagatsu, T., Mogi, M., Ichinose, H., & Togari, A. (2000b). Cytokines in Parkinson's disease. *Journal of Neural Transmission, Supplementum,* 143–151.

Nagatsu, T., & Sawada, M. (2005). Inflammatory process in Parkinson's disease: Role for cytokines. *Current Pharmaceutical Design, 11,* 999–1016.

Nagatsu, T., & Sawada, M. (2006). Cellular and molecular mechanisms of Parkinson's disease: Neurotoxins, causative genes, and inflammatory cytokines. *Cellular and Molecular Neurobiology, 26,* 781–802.

Ogura, K., Ogawa, M., & Yoshida, M. (1994). Effects of ageing on microglia in the normal rat brain: Immunohistochemical observations. *Neuroreport, 5,* 1224–1226.

Ohno, M., Kobayashi, M., Kishi, A., & Watanabe, S. (1997). Working memory failure by combined blockade of muscarinic and beta-adrenergic transmission in the rat hippocampus. *Neuroreport, 8,* 1571–1575.

Ohno, M., & Watanabe, S. (1996). D-cycloserine, a glycine site agonist, reverses working memory failure by hippocampal muscarinic receptor blockade in rats. *European Journal of Pharmacology, 318,* 267–271.

Owen, A. M., Sahakian, B. J., Hodges, J. R., Summers, B. A., Polkey, C. E., & Robbins, T. W. (1995). Dopamine-dependent frontostriatal planning deficits in early Parkinson's disease. *Neuropsychology, 9,* 126–140.

Pawlak, C. R., Ho, Y. J., Schwarting, R. K., & Bauhofer, A. (2003). Relationship between striatal levels of interleukin-2 mRNA and plus-maze behavior in the rat. *Neuroscience letters, 341,* 205–208.

Pawlak, C. R., & Schwarting, R. K. (2006). Striatal microinjections of interleukin-2 and rat behaviour in the elevated plus-maze. *Behavioural Brain Research, 168,* 339–344.

Pawlak, C. R., Schwarting, R. K., & Bauhofer, A. (2005). Cytokine mRNA levels in brain and peripheral tissues of the rat: Relationships with plus-maze behavior. *Molecular Brain Research, 137,* 159–165.

Paxinos, G., & Watson, C. (1986). *The rat brain in stereotaxic coordinates.* London: Academic Press.

Perry, J. C., Hipolide, D. C., Tufik, S., Martins, R. D., Da Cunha, C., Andreatini, R., et al. (2005). Intra-nigral MPTP lesion in rats: Behavioral and autoradiography studies. *Experimental Neurology, 195,* 322–329.

Petitto, J. M., McCarthy, D. B., Rinker, C. M., Huang, Z., & Getty, T. (1997). Modulation of behavioral and neurochemical measures of forebrain dopamine function in mice by species-specific interleukin-2. *Journal of Neuroimmunology, 73,* 183–190.

Petitto, J. M., McNamara, R. K., Gendreau, P. L., Huang, Z., & Jackson, A. J. (1999). Impaired learning and memory and altered hippocampal neurodevelopment resulting from interleukin-2 gene deletion. *Journal of Neuroscience Research, 56,* 441–446.

Piekema, C., Kessels, R. P., Mars, R. B., Petersson, K. M., & Fernandez, G. (2006). The right hippocampus participates in short-term memory maintenance of object-location associations. *Neuroimage, 33,* 374–382.

Reksidler, A. B., Lima, M. M., Zanata, S. M., Machado, H. B., da Cunha, C., Andreatini, R., et al. (2007). The COX-2 inhibitor parecoxib produces neuroprotective effects in MPTP-lesioned rats. *European Journal of Pharmacology, 560,* 163–175.

Sanberg, P. R., Bunsey, M. D., Giordano, M., & Norman, A. B. (1988). The catalepsy test: Its ups and downs. *Behavioral Neuroscience, 102,* 748–759.

Sawada, M., Imamura, K., & Nagatsu, T. (2006). Role of cytokines in inflammatory process in Parkinson's disease. *Journal of Neural Transmission, Supplementum,* 373–381.

Schuster, G. M., & Schmidt, W. J. (1992). D-cycloserine reverses the working memory impairment of hippocampal-lesioned rats in a spatial learning task. *European Journal of Pharmacology, 224,* 97–98.

Sedelis, M., Schwarting, R. K., & Huston, J. P. (2001). Behavioral phenotyping of the MPTP mouse model of Parkinson's disease. *Behavioural Brain Research, 125,* 109–125.

Shen, Y. Q., Hebert, G., Lin, L. Y., Luo, Y. L., Moze, E., Li, K. S., et al. (2005). Interleukine-1beta and interleukine-6 levels in striatum and other brain structures after MPTP treatment: Influence of behavioral lateralization. *Journal of Neuroimmunology, 158,* 14–25.

Sriram, K., Matheson, J. M., Benkovic, S. A., Miller, D. B., Luster, M. I., & O'Callaghan, J. P. (2006). Deficiency of TNF receptors suppresses microglial activation and alters the susceptibility of brain regions to MPTP-induced neurotoxicity: Role of TNF-alpha. *FASEB Journal, 20,* 670–682.

Stanic, D., Finkelstein, D. I., Bourke, D. W., Drago, J., & Horne, M. K. (2003). Time course of striatal re-innervation following lesions of dopaminergic SNpc neurons of the rat. *European Journal of Neuroscience, 18,* 1175–1188.

Stern, Y., Tetrud, J. W., Martin, W. R., Kutner, S. J., & Langston, J. W. (1990). Cognitive change following MPTP exposure. *Neurology, 40,* 261–264.

Sugama, S., Yang, L., Cho, B. P., DeGiorgio, L. A., Lorenzl, S., Albers, D. S., et al. (2003). Age-related microglial activation in 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP)-induced dopaminergic neurodegeneration in C57BL/6 mice. *Brain Research, 964,* 288–294.

Teismann, P., & Schulz, J. B. (2004). Cellular pathology of Parkinson's disease: Astrocytes, microglia and inflammation. *Cell and Tissue Research, 318,* 149–161.

Teismann, P., Tieu, K., Choi, D. K., Wu, D. C., Naini, A., Hunot, S., et al. (2003). Cyclooxygenase-2 is instrumental in Parkinson's disease neurodegeneration. *Proceedings of the National Academy of Sciences, USA of the United States of America, 100,* 5473–5478.

West, W. H., Tauer, K. W., Yannelli, J. R., Marshall, G. D., Orr, D. W., Thurman, G. B., et al. (1987). Constant-infusion recombinant interleukin-2 in adoptive immunotherapy of advanced cancer. *New England Journal of Medicine, 316,* 898–905.

Wood, E. R., Mumby, D. G., Pinel, J. P., & Phillips, A. G. (1993). Impaired object recognition memory in rats following ischemia-induced damage to the hippocampus. *Behavioral Neuroscience, 107,* 51–62.

Wu, D. C., Jackson-Lewis, V., Vila, M., Tieu, K., Teismann, P., Vadseth, C., et al. (2002). Blockade of microglial activation is neuroprotective in the 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine mouse model of Parkinson disease. *Journal of Neuroscience, 22,* 1763–1771.

Xavier, L. L., Viola, G. G., Ferraz, A. C., Da Cunha, C., Deonizio, J. M., Netto, C. A., et al. (2005). 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 Research Protocols, 16,* 58–64.

Yasuda, Y., Shimoda, T., Uno, K., Tateishi, N., Furuya, S., Yagi, K., et al. (2008). The effects of MPTP on the activation of microglia/astrocytes and cytokine/chemokine levels in different mice strains. *Journal of Neuroimmunology, 204,* 43–51.

Zalcman, S., Murray, L., Dyck, D. G., Greenberg, A. H., & Nance, D. M. (1998). Interleukin-2 and -6 induce behavioral-activating effects in mice. *Brain Research, 811,* 111–121.

Zalcman, S. S. (2001). Interleukin-2 potentiates novelty- and GBR 12909-induced exploratory activity. *Brain Research, 899,* 1–9.

Zhang, W. N., Pothuizen, H. H., Feldon, J., & Rawlins, J. N. (2004). Dissociation of function within the hippocampus: Effects of dorsal, ventral and complete excitotoxic hippocampal lesions on spatial navigation. *Neuroscience, 127,* 289–300.

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