行政院國家科學委員會專題研究計畫 成果報告

藥理性調節麩胺酸神經訊遞在 MPTP 所誘發之巴金森氏症動物神經退化及行為缺陷之效果
研究成果報告(精簡版)

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中 文 摘 要 ： 麩胺酸神經系統過度活化會造成興奮性毒性並且參與巴金森氏症之神經退化，
麩胺酸代謝性受體第五亞型（mGluR5）可以調節麩胺酸之神經訊遞功能，因此被認為可能是開發神經保護藥物之作用標的。本研究之目的在於測量 mGluR5 拮抗劑 2-methyl-6-(phenylethylnyl)-pyridine （MPEP）對於 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP)所誘發之巴金森氏症大鼠之神經退化、工作記憶及物件辨識缺損之效果。本實驗使用 Wistar 大鼠為實驗動物，將 MPTP 微量注射到大鼠之中腦黑質體緻密區（substantia nigra pars compacta），以誘發巴金森氏症動物模式，隔天起，動物每天接受腹腔注射 MPEP（2 mg/kg/day, i.p.），連續投藥 14 天。第 8-10 天進行 T-型迷宮試驗以測量工作記憶，第 12-14 天測量物件辨識功能。MPTP 誘發之巴金森氏症動物出現工作記憶缺陷及物件辨識功能缺陷，MPEP 治療可以改善上述兩項行為缺陷，而且，巴金森氏症動物之黑質紋狀體會出現多巴胺神經系統退化，黑質體緻密區內之微膠細胞活（microglia）化增加，海馬迴 CA1 區域出現神經缺損，但是 MPEP 治療可以抑制這些神經組織學上所見之異常。上述結果顯示 mGluR5 在巴金森氏症之生理病理機轉扮演一定之角色，而且 MPEP 可能具有治療巴金森氏症失智之潛力。

中文關鍵詞： 巴金森氏症、失智症、代謝性麩胺酸受體、認知功能

英 文 摘 要 ： Hyperactivity of the glutamatergic system is involved in excitotoxicity and neurodegeneration in Parkinson’s disease (PD). Metabotropic glutamate receptor subtype 5 (mGluR5) modulates glutamatergic transmission and thus has been proposed as a potential target for neuroprotective drugs. The aim of this study was to determine the effects of 2-methyl-6-(phenylethylnyl)-pyridine (MPEP), an mGluR5 antagonist, on working memory, object recognition, and neurodegeneration in a 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP)-induced PD rat model. Male Wistar rats were stereotaxically injected with MPTP into the substantia nigra pars compacta (SNc). Starting 1 day after lesioning (day 1), the rats were treated daily with MPEP (2 mg/kg/day, i.p.) for 14 days and rats underwent a T-maze test on days 8-10 and an object recognition test on days 12-14. MPTP-

lesioned rats showed impairments of working memory in the T-maze test and of recognition function in the object recognition test and both effects were prevented by MPEP treatment. Furthermore, MPTP lesion-induced dopaminergic degeneration in the nigrostriatal system, microglial activation in the SNc, and cell loss in the hippocampal CA1 area were all inhibited by MPEP treatment. These data provide support for a role of mGluR5s in the pathophysiology of PD and suggest that MPEP is a promising pharmacological tool for the development of new treatments for dementia associated with PD.

英文關鍵詞： Parkinson’s disease, dementia, metabotropic glutamate receptor, MPEP, cognition

國科會計畫研究成果報告書

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Role of metabotropic glutamate receptors in cognition and neurodegeneration in an MPTP-induced Parkinson's disease rat model

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Abstract

Hyperactivity of the glutamatergic system is involved in excitotoxicity and neurodegeneration in Parkinson's disease (PD). Metabotropic glutamate receptor subtype 5 (mGluR5) modulates glutamatergic transmission and thus has been proposed as a potential target for neuroprotective drugs. The aim of this study was to determine the effects of 2-methyl-6-(phenylethylnyl)-pyridine (MPEP), an mGluR5 antagonist, on working memory, object recognition, and neurodegeneration in a 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP)-induced PD rat model. Male Wistar rats were stereotaxically injected with MPTP into the substantia nigra pars compacta (SNc). Starting 1 day after lesioning (day 1), the rats were treated daily with MPEP (2 mg/kg/day, i.p.) for 14 days and rats underwent a T-maze test on days 8-10 and an object recognition test on days 12-14. MPTP-lesioned rats showed impairments of working memory in the T-maze test and of recognition function in the object recognition test and both effects were prevented by MPEP treatment. Furthermore, MPTP lesion-induced dopaminergic degeneration in the nigrostriatal system, microglial activation in the SNc, and cell loss in the hippocampal CA1 area were all inhibited by MPEP treatment. These data provide support for a role of mGluR5s in the pathophysiology of PD and suggest that MPEP is a promising pharmacological tool for the development of new treatments for dementia associated with PD.

Keywords: Parkinson's disease, dementia, metabotropic glutamate receptor, MPEP, cognition

1. Introduction

In addition to motor dysfunction, dementia is seen in 25-30% of patients with Parkinson's disease (PD) and is called PD dementia (PDD) (Aarsland et al., 2001; Brown and Marsden, 1984), the main symptoms being deficits in working memory and object discrimination (Barnes et al., 2003; Laatu et al., 2004; Ramirez-Ruiz et al., 2006). However, the development of drug therapy for PDD has been hampered because the pathophysiology is not yet fully understood.

The progressive degeneration of dopaminergic (DAergic) neurons in the substantia nigra pars compacta (SNc) in PD triggers a cascade of functional modifications in basal ganglia circuitry that underlie the motor symptoms. According to the current model of basal ganglia circuitry, the loss of DAergic neurons leads to hyperactivation of the glutamatergic system in the subthalamic nucleus (STN) (Blandini et al., 2000), which provides an excitatory drive onto the SNc (Smith et al., 1996) and the output nuclei of the basal ganglia (Marino et al., 2003), causing a vicious positive feedback loop, i.e., SNc neuronal loss causes STN hyperactivity, which, in turn, causes SNc neuronal loss, thus contributing to the inexorable progression of the neurodegeneration associated with PD (Blandini and Greenamyre, 1998; Marino et al., 2003). Thus, pharmacological blockade of glutamatergic transmission is an effective treatment for PD (Armentero et al., 2006).

Hyperactivity of the glutamatergic system, seen as increased glutamate efflux in the brain, has been observed after nigrostriatal lesioning (Meshul et al., 1999; Robinson et al., 2003). Excessive release of glutamate is an excitotoxic event and is involved in the degeneration of DAergic neurons in PD (Albin and Greenamyre, 1992). Blockade of metabotropic glutamate receptors (mGluRs) is a way of reducing glutamatergic hyperactivity and mGluRs are therefore potential targets for neuroprotective drugs, because they modulate glutamatergic transmission and are implicated in the processes of neurodegeneration and neuroprotection (Bruno et al., 2001). The mGluR5 subtype is abundantly expressed in different brain regions, such as hippocampus, frontal cortex, striatum (Pellegrino et al., 2007; Zhu et al., 2007), and basal ganglia structures, particularly the STN (Testa et al., 1994), and has been proposed as a target for the pharmacological treatment of PD (Rouse et al., 2000).

Acute pretreatment with the mGluR5 antagonist 2-methyl-6-(phenylethylnyl)-pyridine (MPEP) results in neuroprotection of the nigrostriatal DAergic system in the 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP)-induced mouse PD model (Aguirre et al., 2005; Battaglia et al., 2004). Furthermore, 3 weeks of treatment with MPEP alleviates motor deficits in the 6-hydroxydopamine (6-OHDA)-induced PD rat model (Breysse et al., 2003). Since MPTP-lesioned rats exhibit not only neurodegeneration and motor symptoms, but also cognition deficits (Ho et al., 2011; Wang et al., 2010; Wang et al., 2009), it was therefore of interest to examine whether MPEP could alleviate cognition deficits in the animals. The aim of this study was to determine the neuronal and behavioral effects of 2 weeks of MPEP treatment in the MPTP-induced PD rat model.

2. Materials and methods

2.1. Animals

Male Wistar rats (419.5±7.5 g; National Laboratory Animal Center, ROC) were housed in groups of four in acrylic cages (35×56×19 cm) in an animal room on 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 the Care and Use of Laboratory Animals and were approved by the Animal Care Committee of Chung Shan Medical University (IACUC approval No. 1001).

2.2. General procedure

All animals underwent stereotaxic surgery and bilateral infusion into the SNc of MPTP-HCl (1 μmol in 2 μl of saline; Sigma, MO, USA) or vehicle on day 0 (see Surgery section below), as described in our previous reports (Ho et al., 2011; Sy et al., 2010; Wang et al., 2010; Wang et al., 2009). Starting on the day after surgery (day 1), the rats received 14 daily intraperitoneal (i.p.) injections of MPEP (2 mg/kg/day; Sigma, USA) [group name: MPTP+MPEP; n=13] or saline [group name: MPTP+saline; n=14] in a volume of 1 ml/kg at 15:00 h. This dosage was chosen because of a previous report that chronic treatment with MPEP at doses of 1.5 and 3 mg/kg/day for 21 days improves motor deficits in the

6-OHDA-induced PD rat model (Breysse et al., 2003). The rats were subjected to a battery of behavioral tests performed as in our previous studies (Ho et al., 2011; Sy et al., 2010; Wang et al., 2009): a bar test was performed on days 1 and 7, a T-maze test on days 8-10, and an object recognition test on days 12-14. All behavioral tests were started at least 2 h after the beginning of the light phase (7:00 h) and were performed in a dim observation room (28 lux red light) with sound isolation reinforced by a masking white noise of 70 db. The test equipment and objects used in this study were cleaned using 20% ethanol and thoroughly dried before each test trial. On day 15 after MPTP lesioning, the rats were euthanized by exposure to CO_2, transcardially perfused with phosphate-buffered saline, and the brain immediately removed for histological examination.

2.3. Surgery

Brain surgery was performed as described in our previous reports (Ho et al., 2011; Sy et al., 2010; Wang et al., 2010; Wang et al., 2009). Briefly, the rats were anesthetized using Zoletil (20 mg/kg, i.p.; Virbac, Carros, France), then MPTP-HCl (1 μmol in 2 μl of saline) was bilaterally infused into the SNc using the following coordinates adapted from the rat brain atlas (Paxinos G, 1986): AP: -5.0 mm, ML: ±2.0 mm, DV: -7.7 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 [group name: sham+saline; n=12]. Immediately after surgery, the rats were injected intramuscularly with penicillin-G procaine (0.2 ml, 20,000 IU), then housed individually in plastic cages (25 cm × 41 cm × 19 cm) for a week before they were returned to their initial 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

Bar test: The bar test was performed on days 1 and 7 after MPTP lesioning. Catalepsy was evaluated by measuring the mean time (crossing latency) taken for a rat to climb over a 9 cm high bar after being laid across it with its hind limbs on the floor (Sy et al., 2010). Each animal was tested in 3 consecutive trials on each trial day.

T-maze test: The construction of the T-maze and the test procedures were identical to those described in our previous study (Ho et al., 2011). Briefly, in the training session performed on 2 consecutive days (days 8 and 9), the rats learned to find food rewards (chocolate pellets; Kellogg's, Taiwan) in the T-maze using their working memory. On day 10, the percentage of correct responses in a test session was recorded. A training session consisted of 9 trials, each composed of two parts, a forced run and a choice run. In the forced run, one of the arms (left or right according to a random order) was closed by a sliding door and the reward was located at the end of the open arm. In the choice run that was carried out 30 sec after the forced run, both arms were open and the correct response for obtaining a reward was to choose the newly opened arm, the opposite to that used in the forced run. On the following test day, 3 forced-choice-choice run trials were carried out, in which the rats made 2 choices following a single forced run and correct responses in the 6 choice runs were recorded. On the day before T-maze training, the rats were partially food restricted, the diet only being provided for 1 h, while, on the 2 training days, the diet was provided for only 1 h after the behavioral observation on that day. On the test day, food was not provided before testing, but was freely available afterwards.

Object recognition test: The apparatus, an open box (60×60×60 cm), and the test procedure for the object recognition test were identical to those in our previous reports (Ho et al., 2011; Sy et al., 2010; Wang et al., 2009). Each rat was subjected to 3 exposure sessions at 24 h intervals (days 12-14), then, 5 min after the last exposure session, a test session was performed (day 14). Four different objects that were unfamiliar to the rats before the experiment were used for each rat. Three of the objects ("A", "B", and "C") were fixed to the floor 27 cm from three corners of the arena. Starting on day 12 after MPTP lesioning, the rat was allowed to explore the objects in the open box for 5 min on 3 consecutive days, then, 5 min after the last exposure session, object "B" was replaced by a novel object, "D", and the animal was returned to the open box for a 5 min test session. The time spent exploring the objects during the exposure sessions and test session was recorded. Exploration of an object was defined as the rat approaching it and making physical contact with it with its snout and/or forepaws. The percentage of the exploration time spent on object B or D in the sessions [(Time $_{\text{B or D}}$ / Time $_{\text{all objects}}$) × 100%] was calculated. The difference in the percentage of time spent exploring object "B" in exposure 3 and the novel object "D" served as a measure of recognition memory for the familiar object. In addition, rearing number in the test was also recorded. A rearing was recorded when the rat stood on its hind legs, raised both forepaws off

the ground, and stretched its back and was considered to end when at least one forepaw had reached the floor again.

2.5. Histological assessment and image analysis

For histological assessment, on the day 15, 4 or 5 randomly selected rats per group were perfused intracardially with 4% paraformaldehyde in phosphate-buffered saline, then the brains were rapidly removed and post-fixed in 30% sucrose solution containing 4% paraformaldehyde at 4°C until use. To detect DAergic degeneration and microglial activation, frozen coronal brain sections (30 μm) were cut and immunostained at 4°C overnight with mouse monoclonal antibodies against rat tyrosine hydroxylase (TH) (1:2000; Zymade, USA) or rat MHC class II (OX-6; 1:200; BD Biosciences Pharmingen, CA, USA), as in our previous reports (Ho et al., 2011; Sy et al., 2010; Wang et al., 2010; Wang et al., 2009). In sections containing the hippocampus, Nissl staining was used to identify neurons.

The stained brain sections were used to measure histological changes as described previously (Ho et al., 2011; Wang et al., 2010; Wang et al., 2009) using a microscope (ZEISS AXioskop2, Germany) coupled to a CCD (Optronics, USA) and Image Pro Plus Software 6.0 (Media Cybernetics, CA, USA). In this study, we created three square areas of interest, one of 32,037 μm^2 in the striatum to determine the optical density of TH immunoreactivity and one of 2,817,932 μm^2 in the SNc and another of 147,410 μm^2 in the hippocampal CA1 area to determine neuronal density in these regions. In the striatum, we measured the intensity of DAergic projections by converting the TH-stained images to gray-scale, then measuring the gray level of the given area of interest and subtracting the background staining, measured in the non-immunoreactive corpus callosum; thus, the relative optical density was restricted to the values generated by the TH-reactive tissue. In the SNc, we measured the density of DAergic neurons and activated microglia by capturing images, overlaying an area of interest in this region, and counting the somas of TH-immunoreactive neurons and activated microglia in these areas. In the hippocampal CA1 area, as the neurons were tightly packed, it was difficult to directly count the number of pyramidal neurons from a 30 μm thick brain section, so we measured the density of pyramidal neurons by estimating neuronal density using a semi-quantitative method involving calculating the percentage of an area of interest in the CA1 area occupied by Nissl-stained neurons. Although a stereological approach involving the counting of cells in a complete series of sections would provide additional data (Ferro et al.,

2005), calculating the cell number in representative brain sections yielded similar histological results to those reported in the literature (Da Cunha et al., 2001). All the thickness of the brain sections, location of immunostained area, and total number of cells in this area affect the levels of immunoreactivity (Xavier et al., 2005), which could influence the accuracy of image analysis of densitometry. For avoiding inconsistency happen between the brain sections of different groups, in the current study the representative brain sections were taken according to and the location of areas used for measuring neuronal density were based on the atlas of rat brain (Paxinos and Watson, 1986), and, in addition, immunohistological reactions of these sections were performed at the same time.

2.6. Data analysis

Analysis of variance (ANOVA), followed by the least-significant difference (LSD) post hoc test, was used to analyze the bar test and T-maze test results and the paired-samples *t*-test was used to analyze the object recognition test data. All results are expressed as the mean±SEM. The level of significance was defined as $P<0.05$ (two-tailed).

3. Results

ANOVA revealed that, on day 1 after surgery, the crossing latency in the bar test was different between the groups ($F(2,24)=5.82$, $P<0.01$). The LSD post hoc test showed that the crossing latency was significantly longer in rats that had undergone MPTP lesioning (groups MPTP+saline and MPTP+MPEP) than in the sham+saline group (both P values<0.01), indicating that MPTP lesioning induced motor impairment in the PD model. However, on day 7 after surgery, no significant difference was observed between the groups, indicating spontaneous recovery of motor function, as in our previous reports (Ho et al., 2011; Sy et al., 2010; Wang et al., 2010; Wang et al., 2009) (Fig. 1).

ANOVA indicated that there were differences in the percentage of correct responses in the T-maze test between the groups ($F(2,38)=9.55$, $P<0.001$). The LSD post hoc test showed that MPTP lesioning significantly decreased the percentage of correct responses in the T-maze test compared to the sham-operated group ($P<0.001$), indicating a deficit of working memory. There was no significant difference in the percentage of correct responses between

the rats in the sham-operated group and those receiving MPEP treatment after MPTP lesioning (Fig. 2).

The procedure used in the object recognition test is shown in Fig. 3A. ANOVA revealed that there were no significant differences between the groups in total exploration time and the percentage of time spent exploring object "B" in the 3 exposure sessions (data not shown). As shown in Fig. 3B, analysis using the paired-samples *t*-test showed that the sham+saline group (df=11, t=4.26, P=0.001) and the MPTP+MPEP group (df=12, t=3.40, P=0.005) spent a higher percentage of time exploring object "D" than exploring object "B", whereas the MPTP+saline group did not. In terms of rearing number in the object recognition test, ANOVA with repeated measures revealed no significant treatment effect, time effect, or time-by-treatment interaction (Fig. 3C).

Representative photomicrographs of immunostained and Nissl-stained brain section are shown in Figs. 4-7. TH immunoreactivity was observed in the cell bodies of DAergic neurons in the SNc (Fig. 4) and in DAergic processes in the striatum (Fig. 5). ANOVA showed that rats in the MPTP+saline group exhibited a decreased density of DAergic neurons in the SNc (F(2,12)=17.73, P<0.001) (Figs. 4B and E) and a lower relative optical density of TH immunoreactivity in the striatum (F(2,12)=43.26, P<0.001) (Figs. 5B and E) than the sham+saline group. MPEP treatment prevented the MPTP-induced decrease in the density of DAergic neurons in the SNc (Figs. 4C and E) and inhibited the MPTP-induced decrease in TH immunoreactivity in the striatum (Figs. 5C and E). Activated microglia, indicated by accumulation of OX-6-positive cells, were detected in the SNc in the MPTP+saline group (Fig. 6B), but not in the sham+saline group or the MPTP+MPEP group (Figs. 6A and C). ANOVA showed that the density of activated microglia in the MPTP+saline group was higher than that in the sham+saline group (F(2,11)=173.26, P<0.001) and that MPEP treatment prevented MPTP-induced microglial activation (Fig. 6E). In addition, the neuronal density in the pyramidal cell layer in the hippocampal CA1 area was decreased in the MPTP+saline group compared to the sham-operated group (F(2,11)=12.31, P=0.003) and this effect was inhibited in the MPTP+MPEP group.

4. Discussion

In the present study, MPTP lesioning caused behavioral deficits in working memory and object recognition. A two week period of treatment with MPEP, an mGluR5 antagonist, at the daily dosage of 2 mg/kg/day prevented the above behavioral deficits. MPTP lesioning also decreased the density of DAergic neurons in the SNc and of pyramidal neurons in the hippocampal CA1 area and induced microglia activation in the SNc. The above neurohistological and neuroinflammatory changes were inhibited by MPEP treatment. To our knowledge, this is the first evidence that MPEP can prevent hippocampal cell loss in a PD rat model. Moreover, consistent with our previous reports (Ho et al., 2011; Wang et al., 2010; Wang et al., 2009), MPTP-lesioned rats showed cognition deficits accompanied by neurodegeneration in the nigrostriatal system and hippocampus, and thereby may model the symptoms and pathophysiology of PDD. These results suggest that blocking mGluR5 may have beneficial effects on neuronal and behavioral impairments in PDD.

In line with our previous report, catalepsy was observed after MPTP lesioning, but not sham operation, and this maintained for around 4-5 days and recovered at day 7 (Ho et al., 2011; Sy et al., 2010). Spontaneous motor recovery was observed in all of the MPTP-lesioned rats, irrespective of whether they received MPEP treatment or not. Motor recovery was further supported by the lack of a difference between the groups in rearing number in the object recognition test, suggesting that behavioral performance in the tests was not confounded by gross motor impairment or general sickness. When comparing the MPTP-induced and other PD models, for example, 6-OHDA lesion model, the MPTP model would seem more favorable as it produces a bilateral dopamine lesion, similar to the slow onset of idiopathic PD (Potashkin et al., 2011), whereas the 6-OHDA model is classically an unilateral lesion (Iancu et al., 2005). In the unilateral model, it takes around 2 weeks to cause full dopamine lesion after injection of 6-OHDA into the medial forebrain bundle, which may mimic the progressive loss of neurons seen in PD. The MPTP bilateral lesion model is considered more relevant to PD since both hemispheres are dopamine depleted and they may have more specificity towards behavioral impairments (Potashkin et al., 2011), including, but not limited to, cognitive dysfunctions, as demonstrated in the current study. However, the MPTP model fails to encompass the wide assortment of motor impairments seen in PD patients.

Sub-chronic treatment with MPEP prevented the MPTP-induced deficits in working memory and object recognition. In the T-maze test, the rat has to learn a rule to make the

correct choice to obtain a reward in the test run, in which the food was located in the arm that was closed in the previous forced run. Thus, performance in the T-maze test is regarded as working memory because the location of the reward pellets is trial-dependent (Ando et al., 2002). In the present study, the sham-operated rats showed around 80% of correct responses, significantly higher than chance (50%). MPTP lesioning significantly decreased the percentage of correct responses in the T-maze test, indicating impairment of working memory. Previous studies have also found that MPTP-lesioned rats show disturbances of working memory (Bellissimo et al., 2004; Braga et al., 2005; Ho et al., 2011; Hsieh et al., 2012) and episodic-like memory (Wang et al., 2010) and impairments of learning and memory in the two-way active avoidance test (Da Cunha et al., 2001; Ferro et al., 2005; Gevaerd et al., 2001), and have thus suggested these rats can model PD amnesia. Rats in the object recognition test have a natural tendency to spend more time exploring novel, rather than familiar, objects when there are two choices, reflecting discrimination between novel and old objects (Ennaceur and Delacour, 1988). In the present study, the MPTP-lesioned rats did not show this phenomenon, indicating a deficit in object recognition. Although the value of percentage of time spent exploring new object seems higher than that spent exploring old object, there was no significance. This result was in line with our previous reports (Ho et al., 2011; Hsieh et al., 2012; Sy et al., 2010; Wang et al., 2009). Two weeks of MPEP treatment prevented the MPTP-induced deficits in working memory and object recognition. Similarly, a previous report showed that an 8-day treatment of MPEP at the dose of 3 mg/kg/day antagonized visuo-spatial discrimination deficit induced by bilateral 6-OHDA lesioning of the striatum in mice (De Leonibus et al., 2009). These results suggest that mGluR5s may be involved in the cognitive impairment in PD.

Impairments of memory and recognition, the cardinal symptoms of PDD, might result from dysfunction of the hippocampus, as this brain area is involved in spatial navigation (Zhang et al., 2004), visual recognition, recognition memory (Broadbent et al., 2004), and short-term memory associating an object and its location (Li and Chao, 2008; Piekema et al., 2006). Moreover, the hippocampal CA1 area is responsible for temporal and working memory (Hunsaker et al., 2006) and for recognizing the spatial arrangement of objects (Wan et al., 1999). The present study showed that MPTP lesioning significantly suppressed working memory and object recognition and that these behavioral impairments were accompanied by cell loss in the hippocampal CA1 area. Interestingly, MPEP treatment prevented both the behavioral changes and the cell loss. Moreover, DAergic degeneration in the nigrostriatal

system causes an increase of ligand binding to mGluR5 in the hippocampus (Zhu et al., 2007). We therefore suggest that there may be an up-regulation of mGluR5 in the hippocampus of the current PD model and this change may thus lead to glutamatergic dysfunction and be involved in the cognition impairment and cell loss in the hippocampus.

A previous study demonstrated the presence of activated microglia in PD brains and suggested that these cells are involved in the neurodegenerative process (McGeer et al., 1988). Another animal study demonstrated that microglial activation occurs in the brain after MPTP lesioning (Kohutnicka et al., 1998), while others showed that activated microglia are seen in the SNc of rats at two weeks after lesioning (Ho et al., 2011; Sy et al., 2010; Wang et al., 2010), indicating that neurodegeneration leads to microglia activation. In addition, activated microglia release inflammatory cytokines (Yasuda et al., 2008), which may lead to cell death (Nakajima and Kohsaka, 2004) and aggravate neuroinflammation and thus play an important role in the pathophysiology of PD (McGeer and McGeer, 2004). In the present study, MPTP lesioning caused microglial activation in the SNc, which was prevented by MPEP treatment. Parallely, MPTP-induced DAergic degeneration in the SNc and striatum was also abolished by MPEP treatment, indicating a correlation between microglial activation and neurodegeneration.

Glutamate, an excitatory neurotransmitter abundantly distributed in the central nervous system, can activate NMDA receptors and mGluR5s (Daw et al., 1993). Hyperactivation of the glutamatergic system has been reported to play a critical role in the neuronal and behavioral symptoms in the PD rat model (Battaglia et al., 2004). Degeneration of DAergic system induced by 6-OHDA lesioning results in an up-regulation of mGluR5 in the striatum (Zhu et al., 2007). Stimulation of mGluR5 facilitates glutamate release in the striatum (Rodrigues et al., 2005). MPTP lesioning has also been reported to increase glutamate release in the striatum (Robinson et al., 2003), cause hyper-glutamatergic activity in the STN (Mosley et al., 2006), and result in excitotoxicity (Mosley et al., 2006; Plaitakis and Shashidharan, 2000). These changes may be involved in neuroinflammation and cell loss in the DAergic system and hippocampus in PD brains (Imamura et al., 2003). Since that NMDA receptors are densely distributed in some brain regions, for example, the striatum and hippocampus (Monaghan and Cotman, 1985), and that mGluR5s are abundantly expressed in basal ganglia structures, particularly the STN (Testa et al., 1994), glutamatergic dysfunction induced by MPTP may underlie the neurodegeneration in the SNc, striatum, and hippocampus and may

explain the behavioral deficits seen in MPTP-lesioned rats. Furthermore, there are reciprocal synergistic interactions between mGluR5s and NMDA receptors (Turle-Lorenzo et al., 2005). Activation of mGluR5s positively modulates NMDA receptors by relieving the Mg^{2+} blockade of NMDA receptors (Bruno et al., 2001). Stimulation of mGluR5s causes excitation of neurons in the STN that further potentiates NMDA-induced activation of STN neurons (Awad et al., 2000). In addition, activation of NMDA receptors amplifies the activity of mGluR5s by preventing receptor desensitization (Alagarsamy et al., 1999). Although the use of compounds blocking NMDA receptors has been demonstrated to result in an improvement in motor activity (St-Pierre and Bedard, 1995), cognitive function (Hsieh et al., 2012), and survival of nigrostriatal DAergic neurons in the PD rat model (Ferro et al., 2007; Turski et al., 1991; Zuddas et al., 1992), the therapeutic potential of NMDA antagonists is substantially hampered by the occurrence of significant neurological side effects. Thus, compounds acting on mGluR5s show promise for the treatment of PD (Marino et al., 2003).

There are several potential mechanisms and possible brain sites at which MPEP might have acted after its systemic administration. More likely, MPEP could modulate glutamate release. Previous reports have shown that lesions of the nigrostriatal DAergic system by 6-OHDA in rats caused mGluR5 up-regulation in different brain regions, such as hippocampus, frontal cortex, and striatum (Pellegrino et al., 2007; Zhu et al., 2007). Decreasing glutamate release and its excitotoxic consequence is likely a mechanism by which MPEP exerts neuroprotective function in the DAergic system and hippocampus because MPEP can block glutamate release induced by mGluR5 stimulation (Rodrigues et al., 2005). Moreover, mGluR5 blockade in the nuclei of the basal ganglia may also be responsible for the effects of MPEP. The STN is a key nucleus in the basal ganglia that provides the major glutamatergic excitatory input to the basal ganglia output nuclei. The STN plays a critical role in motor function as well as in the pathophysiology of PD (Marino et al., 2003). Immunohistochemical studies at the light and electron microscopic levels indicate that mGluR5s are localized in the neurons of STN and of substantia nigra pars reticulate (SNr); activation of mGluR5 causes depolarization of neurons in the STN (Awad et al., 2000) and excitation of SNr projection neurons (Marino et al., 2001), indicating that mGluR5 plays an important role in excitatory control of STN on motor circuit in the basal ganglia. It has been shown that akinetic deficits in 6-OHDA lesion-induced PD rat model are associated with increased neuronal metabolic activity in the STN and SNr. The above akinesia and increase of nuclei activity are alleviated by a 3-week chronic treatment with MPEP at the doses of 1.5

and 3 mg/kg/day (Armentero et al., 2006; Breysse et al., 2003; Breysse et al., 2002; Turle-Lorenzo et al., 2005). These results suggest that MPEP, through blocking mGluR5, may reduce the overactivity of STN neurons, decrease excitatory drive in the basal ganglia, and limit the excitotoxicity associated with hyperactivity of glutamatergic system (Blandini, 2001) and may thus result in symptomatic relief in PD. The present study provided further support for the above hypothesis by showing that sub-chronic systemic MPEP treatment inhibited the cognitive deficits and neuronal degeneration in the MPTP-induced PD rat model. Our data agree with a report showing protective effects of MPEP against MPTP-induced neurotoxicity in the nigrostriatal DAergic system (Aguirre et al., 2005).

The important role of mGluR5s in synaptic plasticity, learning, and memory (Balschun and Wetzel, 2002; Petersen et al., 2002) may raise concerns about using MPEP for the treatment of dementia in PD, but no adverse side effects were observed in the present study. In addition, a previous study also showed that both acute and sub-chronic administrations of MPEP at the dosage of 3 mg/kg/day do not affect the function of visuo-spatial discrimination in control mice (De Leonibus et al., 2009). Administration of MPEP to patients with PD might therefore have both symptomatic and neuroprotective effects (Blandini and Greenamyre, 1998; Blandini et al., 2000). Further, although the acute manifestations of DAergic lesion and behavioral changes in the MPTP-treated rats are different from the progression of neurodegeneration and onset of symptoms with time seen in PD patients, MPTP-induced DAergic degeneration maintains for a long period (Sy et al., 2010), which may replicate the late course of PD. Thus, an effective treatment for preventing neuronal loss and behavioral deficits in this model may imply a potential of application in PD. Nevertheless, caution is required when applying data from animal studies to humans (Potashkin et al., 2011). Finally, a single dose, 2 mg/kg/day, of MPEP was used in the present study, so we do not know whether different results would have been obtained if higher or lower doses of MPEP had been used.

In summary, the present study shows that sub-chronic administration of MPEP inhibits MPTP-induced deficits in working memory and object recognition and suppresses neuroinflammation and neurodegeneration in the DAergic system and hippocampal CA1 area. These data provide support for a role for mGluR5s in the pathophysiology of PD and suggest that MPEP is a promising pharmacological tool for the development of new treatments for PDD.

Acknowledgements

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Figure legends

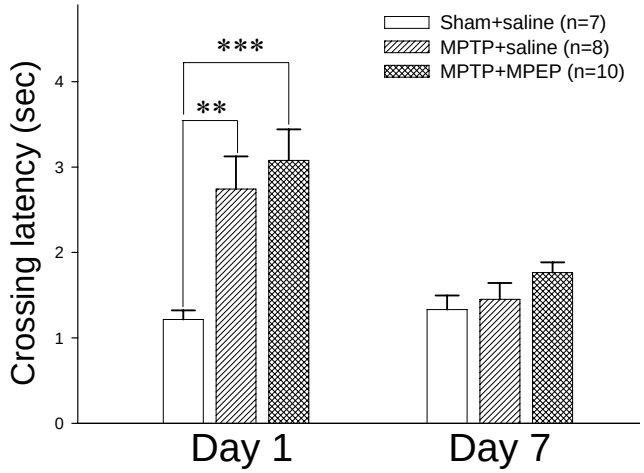

Fig. 1. Effects of MPEP on catalepsy in MPTP-lesioned rats in the bar test. MPTP (1 μmol) was bilaterally infused into the substantia nigra pars compacta, then MPEP (2 mg/kg/day, i.p.) or saline (1 ml/kg/day, i.p.) was administered from day 1 after MPTP lesioning for 14 days. The bar test was performed on days 1 and 7 after MPTP lesioning. The data are expressed as the mean±SEM for the indicated number of rats. ** P<0.01, *** P<0.001 compared to the sham-operated controls.

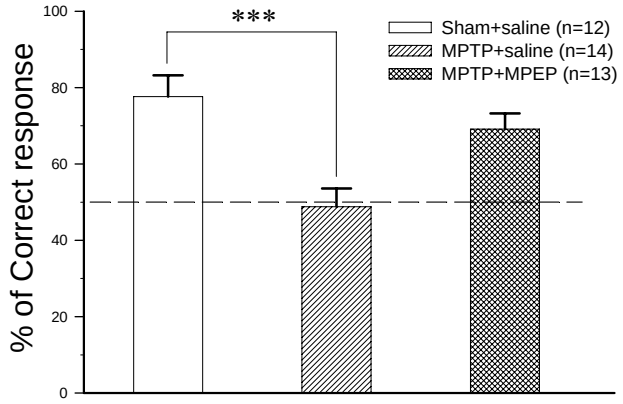

Fig. 2. Effects of MPEP on the behavior of MPTP-lesioned rats in the T-maze test. Animals were treated as in Fig. 1 and the T-maze test was performed on day 10 after surgery. The data are expressed as the mean±SEM. *** P<0.001 compared to the sham+saline group.

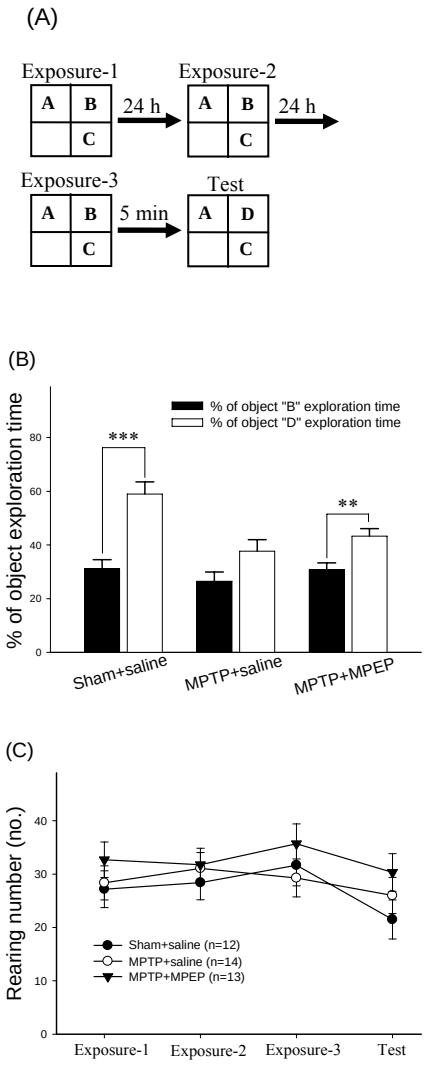

Fig. 3. Effects of MPEP on object recognition in MPTP-lesioned rats. Animals were treated as in Fig. 1 and the object recognition test was performed on days 12–14 after surgery. (A) Schematic diagram of the arrangement of the objects in the test. Rats underwent 3 exposure sessions (5 min each) at 24 h intervals, then were tested for 5 min starting 5 min after the end of exposure session 3. In the test session, object "B" was replaced by a novel object "D". (B) Percentage of time spent exploring object "B" or "D". (C) Rearing number in the exposure and test sessions. The data are expressed as the mean±SEM. ** $P<0.01$, *** $P<0.001$ compared to the percentage of time spent exploring object "B" (paired *t*-test).

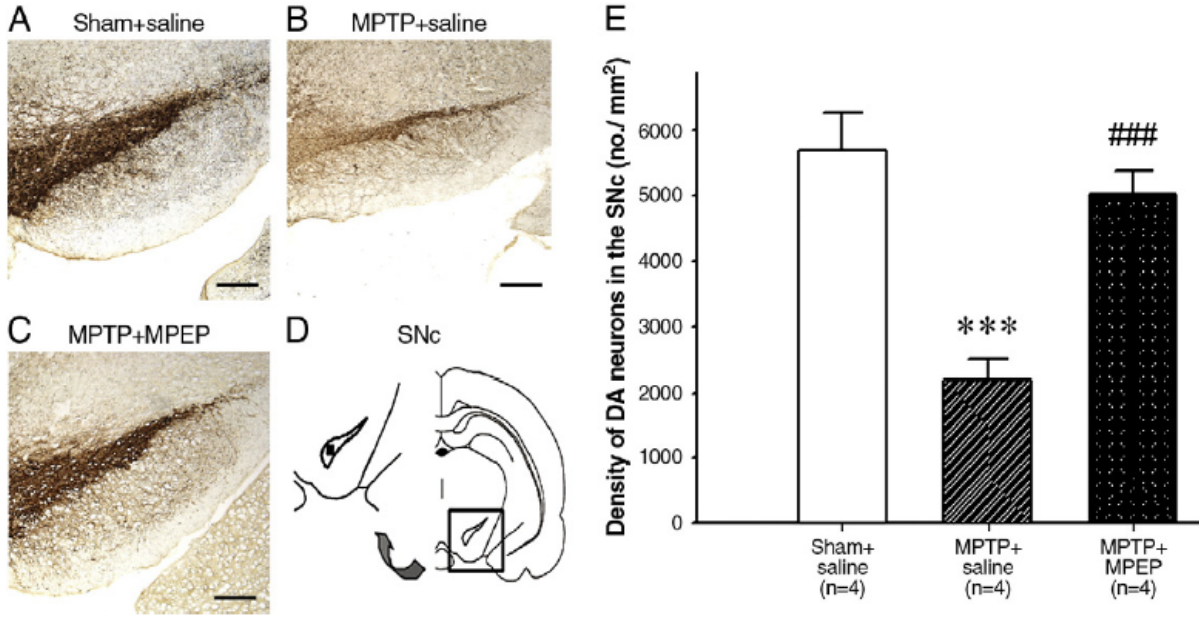

Fig. 4. Effects of MPEP on the MPTP-induced change in dopaminergic neurons in the SNc on day 15 after surgery. Animals were treated as in Fig. 1. Dopaminergic neurons stained for tyrosine hydroxylase are shown in representative coronal sections. Magnification, 50×; bar, 200 μm. The black square in the schematic drawing indicates the area used for measuring the density of dopaminergic neurons. *** $P<0.001$ compared to the sham+saline group. ### $P<0.001$ compared to the MPTP+saline group.

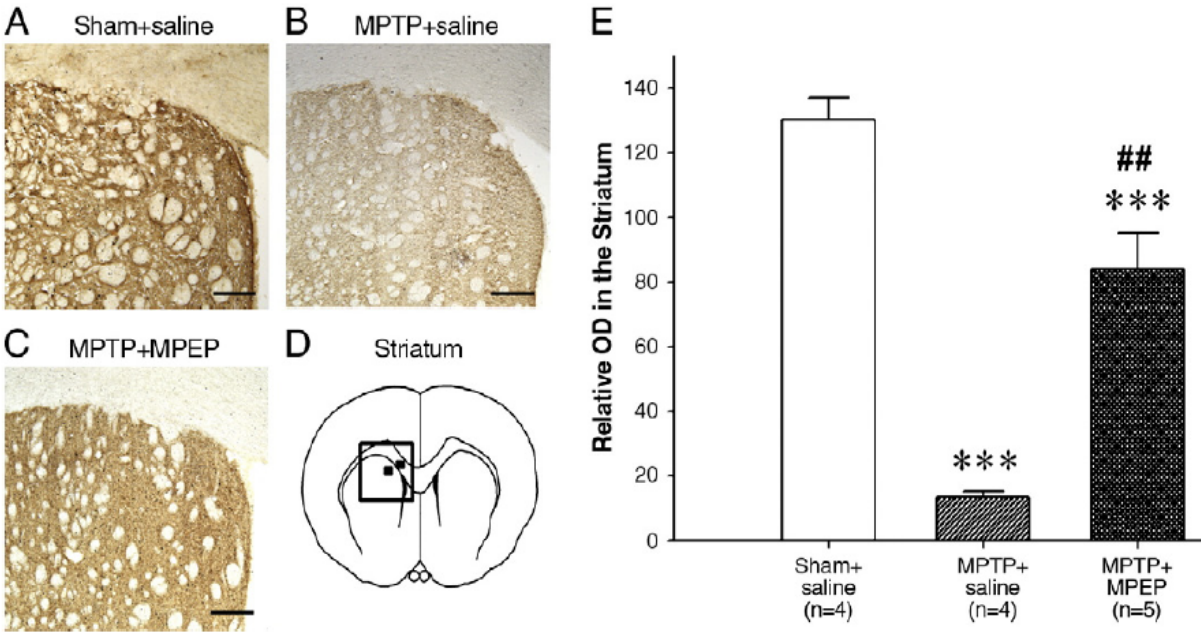

Fig. 5. Effects of MPEP on the MPTP-induced change in tyrosine hydroxylase immunoreactivity in the striatum on day 15 after surgery. Animals were treated as in Fig. 1. Magnification, 50×; bar, 200 μm. The black square in the schematic drawing indicates the area used for measuring the optical density (OD). *** $P<0.001$ compared to the sham+saline group. ## $P<0.01$ compared to the MPTP+saline group.

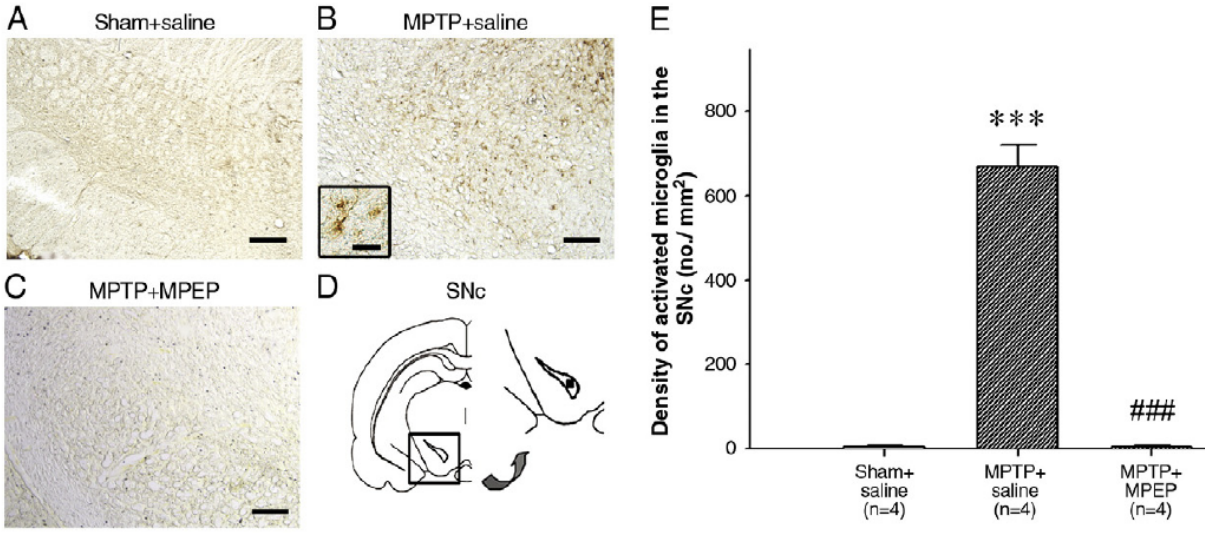

Fig. 6. Effects of MPEP on the MPTP-induced activation of microglia in the SNc on day 15 after surgery. Animals were treated as in Fig. 1. Magnification, 50×; bar, 200 μm. A high magnification image (200×, bar, 20 μm) of activated microglia is shown in the inset. The black square in the schematic drawing indicates the area used for measuring the density of activated microglia in the SNc. *** $P<0.001$ compared to the sham+saline group. ### $P<0.001$ compared to the MPTP+saline group.

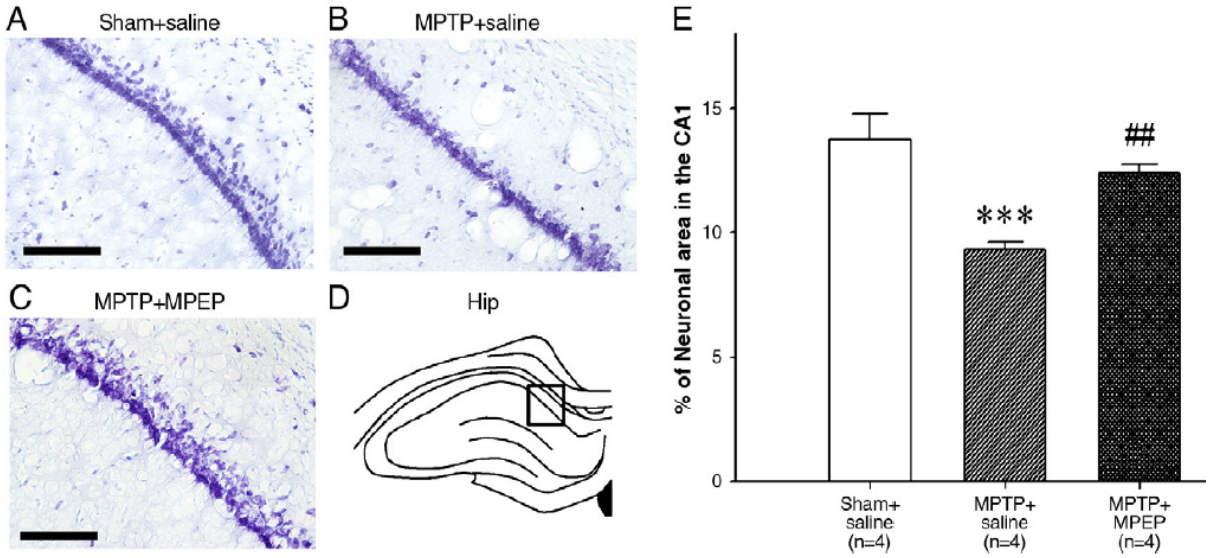

Fig. 7. Effects of MPEP on the MPTP-induced cell loss in the hippocampal CA1 area on day 15 after surgery. Animals were treated as in Fig. 1. The images show Nissl-stained pyramidal neurons in the CA1 area of the hippocampus (Hip), as indicated in the square in the schematic drawing. Magnification, 200×; bar, 100 μm. *** $P<0.001$, compared to the sham+saline group, ## $P<0.01$, compared to the MPTP+saline group.

國科會補助計畫衍生研發成果推廣資料表

日期:2012/11/15

國科會補助計畫	計畫名稱: 藥理性調節麩胺酸神經訊遞在MPTP所誘發之巴金森氏症動物神經退化及行為缺陷之效果
	計畫主持人: 何應瑞
	計畫編號: 100-2410-H-040-003- 學門領域: 生物心理學

無研發成果推廣資料

100 年度專題研究計畫研究成果彙整表

計畫主持人：何應瑞	計畫編號：100-2410-H-040-003-
計畫名稱：藥理性調節麩胺酸神經訊遞在 MPTP 所誘發之巴金森氏症動物神經退化及行為缺陷之效果	

成果項目			量化			單位	備註（質化說明：如數個計畫共同成果、成果列為該期刊之封面故事...等）
			實際已達成數（被接受或已發表）	預期總達成數(含實際已達成數)	本計畫實際貢獻百分比		
國內	論文著作	期刊論文	0	0	100%	篇	
		研究報告/技術報告	1	0	100%		廖丹瑜、洪櫻慈、周璟言、黃冠達、何詩君、黃國洲、張思毅、徐詩惠、吳聲輝、廖娟妙、何應瑞＊。增加麩胺酸轉運子之表現對巴金森氏症大鼠的認知缺陷之效果。中華民國生醫材料及藥物致放學會 2012 年會論文集，Apr., 2012, Taipei
		研討會論文	2	0	100%		1. Hsu SH, SC Ho, GJ Huang, SH Wu, CY Chou, YC Hung, TY Liao, GD Huang, SY Chang, YJ Ho*. Effects of modulation GLT-1 expression on cognition deficits in Parkinson's disease rat model. 第 27 屆生物醫學年會，Mar. 17-18, 2012, 台北 2. Huang GJ, SC Ho,

							disease animal model. Taiwanese Psychology Association 50th Conference, Oct. 15-16, 2011, Tai-Chung, Taiwan.
		專書	0	0	100%		
	專利	申請中件數	0	0	100%	件	
		已獲得件數	0	0	100%		
	技術移轉	件數	0	0	100%	件	
		權利金	0	0	100%	千元	
	參與計畫人力（本國籍）	碩士生	1	0	100%	人次	
		博士生	0	0	100%		
		博士後研究員	0	0	100%		
		專任助理	0	0	100%		
國外	論文著作	期刊論文	4	0	100%	篇	本計畫之成果主要發表 2 篇 SCI 論文: 1. Pharmacology, Biochemistry and Behavior 102: 64-71, 2012； 2. Behav Brain Res 229(1): 41-47, Jan. 10, 2012. 另,運用本計畫經費挹注發表 2 篇 SCI 論文： 1. Kaohsiung J Med Sci 28(8): 407-17, Aug. 16, 2012； 2. Chin J Physiol 55(4): 245-252, Aug. 31, 2012
		研究報告/技術報告	0	0	100%		
		研討會論文	2	0	100%		1. Ho YJ*, YC Hung, CY Chou, SC Ho, GJ Huang, TY Liao, GD Huang, SH Hsu, SH Wu. Glutamatergic blockades diminish

							in Parkinson's disease rat model . Bilateral Russian-Taiwanese Seminar Jul 2, 2012. Novosibirsk, Russia. 2. Ho YJ*, YC Hung, CY Chou, SC Ho, GJ Huang, TY Liao, GD Huang, SH Hsu, SH Wu. Effects of glutamatergic blockade on behavioral and neuronal changes in Parkinson's disease rat model. The 7th Conference of Siberian Physiologists. Jun. 27-29, 2012, Krasnoyarsk, Russia
		專書	0	0	100%	章/本	
	專利	申請中件數	0	0	100%	件	
		已獲得件數	0	0	100%		
	技術移轉	件數	0	0	100%	件	
		權利金	0	0	100%	千元	
	參與計畫人力（外國籍）	碩士生	1	0	100%	人次	
		博士生	0	0	100%		
		博士後研究員	0	0	100%		
		專任助理	0	0	100%		
其他成果 (無法以量化表達之成果如辦理學術活動、獲得獎項、重要國際合作、研究成果國際影響力及其他協助產業技術發展之具體效益事項等，請以文字敘述填列。)		計畫主持人在 2012 年 5 月 3-8 日與俄羅斯科學院學者（Prof. Amstislavskaya 等人）在計畫執行學校（中山醫學大學）進行交流時，報告此研究計畫之成果，獲得熱烈迴響，並獲邀前往俄羅斯克拉司諾雅（Krasnoyarsk）醫學大學出席第 7 屆西伯利亞生理學家會議報告研究成果，於會議中又有其他俄羅斯學者邀請計畫主持人安排日期前往其任教學校發表演講。下列是在俄羅斯所發表之成果： 1. Ho YJ*, YC Hung, CY Chou, SC Ho, GJ Huang, TY Liao, GD Huang, SH Hsu, SH Wu. Glutamatergic blockades diminish behavioral and neuronal deficits in Parkinson's disease rat model . Bilateral Russian-Taiwanese Seminar Jul 2, 2012. Novosibirsk, Russia. 2. Ho YJ*, YC Hung, CY Chou, SC Ho, GJ Huang, TY Liao, GD Huang, SH Hsu, SH Wu. Effects of glutamatergic blockade on behavioral and neuronal changes in Parkinson's disease rat model. The 7th Conference of Siberian					

	Physiologists. Jun. 27-29, 2012, Krasnoyarsk, Russia

	成果項目	量化	名稱或內容性質簡述
科教處計畫加填項目	測驗工具(含質性與量性)	0	
	課程/模組	0	
	電腦及網路系統或工具	0	
	教材	0	
	舉辦之活動/競賽	0	
	研討會/工作坊	0	
	電子報、網站	0	
	計畫成果推廣之參與（閱聽）人數	0	

國科會補助專題研究計畫成果報告自評表

請就研究內容與原計畫相符程度、達成預期目標情況、研究成果之學術或應用價值（簡要敘述成果所代表之意義、價值、影響或進一步發展之可能性）、是否適合在學術期刊發表或申請專利、主要發現或其他有關價值等，作一綜合評估。

1. 請就研究內容與原計畫相符程度、達成預期目標情況作一綜合評估

■達成目標

□未達成目標（請說明，以 100 字為限）

□實驗失敗

□因故實驗中斷

□其他原因

說明：

2. 研究成果在學術期刊發表或申請專利等情形：

論文：■已發表 □未發表之文稿 □撰寫中 □無

專利：□已獲得 □申請中 ■無

技轉：□已技轉 □洽談中 ■無

其他：(以 100 字為限)

運用本計畫經費所得之研究成果已經發表之論文如下：

1. Pharmacology, Biochemistry and Behavior 102: 64-71, 2012. (SCI)
2. Kaohsiung J Med Sci 28(8): 407-17, Aug. 16, 2012. (SCI)
3. Chin J Physiol 55(4): 245-252, Aug. 31, 2012. (SCI)
4. Behav Brain Res 229(1): 41-47, Jan. 10, 2012 (SCI)

3. 請依學術成就、技術創新、社會影響等方面，評估研究成果之學術或應用價值（簡要敘述成果所代表之意義、價值、影響或進一步發展之可能性）(以 500 字為限)

本項基礎研究之成果顯示：麩胺酸神經系統過渡活化參與巴金森氏症之神經退化機轉，使用藥理性操弄降低麩胺酸神經訊遞活性，可以抑制巴金森氏症之神經退化及認知功能障礙之症狀，此研究成果將有利於治療巴金森氏症之藥物設計。研究摘要如下：

Hyperactivity of the glutamatergic system is involved in excitotoxicity and neurodegeneration in Parkinson's disease (PD). Metabotropic glutamate receptor subtype 5 (mGluR5) modulates glutamatergic transmission and thus has been proposed as a potential target for neuroprotective drugs. The aim of this study was to determine the effects of 2-methyl-6-(phenylethylnyl)-pyridine (MPEP), an mGluR5 antagonist, on working memory, object recognition, and neurodegeneration in a 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP)-induced PD rat model. Male Wistar rats were stereotaxically injected with MPTP into the substantia nigra pars compacta (SNc). Starting 1 day after lesioning (day 1), the rats were treated daily with MPEP (2 mg/kg/day, i.p.) for 14 days and rats underwent a T-maze test on days 8-10 and an object recognition test on days 12-14. MPTP-lesioned rats showed

impairments of working memory in the T-maze test and of recognition function in the object recognition test and both effects were prevented by MPEP treatment. Furthermore, MPTP lesion-induced dopaminergic degeneration in the nigrostriatal system, microglial activation in the SNc, and cell loss in the hippocampal CA1 area were all inhibited by MPEP treatment. These data provide support for a role of mGluR5s in the pathophysiology of PD and suggest that MPEP is a promising pharmacological tool for the development of new treatments for dementia associated with PD.