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# An application of a new planar positron imaging system (PPIS) in a small animal: MPTP-induced parkinsonism in mouse

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***Objective:*** Recent animal PET research has led to the development of PET scanners for small animals. A planar positron imaging system (PPIS) was newly developed to study physiological function in small animals and plants in recent years. To examine the usefulness of PPIS for functional study in small animals, we examined dopaminergic images of mouse striata in MPTP-induced parkinsonism. ***Methods:*** Male C57BL/6NCrj mice were treated with MPTP 7 days before the PPIS study. Scans were performed to measure dopamine $D_1$ receptor binding and dopamine transporter availability with [$^{11}$C]SCH23390 (about 2 MBq) and [$^{11}$C]$\beta$-CFT (about 2 MBq), respectively. After the PPIS study, dopamine content in the striatum was measured by HPLC. ***Results:*** The MPTP treatment significantly reduced dopamine content in the striatum 7 days after treatment. In the MPTP-treated group, [$^{11}$C]$\beta$-CFT binding in the striatum was significantly decreased compared with the control group, while striatal [$^{11}$C]SCH23390 binding was not affected. Dopamine content in the striatum was significantly correlated with the striatal binding of [$^{11}$C]$\beta$-CFT. ***Conclusion:*** The present results suggest that PPIS is able to determine brain function in a small animal. Using PPIS, high throughput imaging of small animal brain functions could be achieved.

**Key words:** planar positron imaging system, mouse, MPTP, [$^{11}$C]SCH23390, [$^{11}$C]$\beta$-CFT

## INTRODUCTION

RECENT ANIMAL PET research has led to the development of a PET scanner, a so-called micro-PET,[1–4] for small animals such as rats and mice, because several kinds of disease model are easily prepared in small animals. Use of transgenic or knockout mice facilitates investigation of functional changes in a specific gene. PET research in small animals makes it possible to decrease the numbers of animals used in one experiment and may also be useful in new drug discovery.

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In recent years, a new planar positron imaging system (PPIS) whose spatial resolution is less than 2.1 mm FWHM has been developed to study small animals and plants (Fig. 1).[5] This system enables imaging of the two-dimensional (projection) distribution of a positron emitter in real time.[5] The aim of the present study was to examine whether this system enabled imaging of the brain function in the mouse. As an application, we examined the changes of dopaminergic function in a mouse model of Parkinson's disease.

An animal model of Parkinson's disease induced by a neurotoxin, 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP), has often been used in mice and non-human primates.[6–10] MPTP was also reported to produce acute parkinsonism in humans.[11] A metabolite of MPTP, 1-methyl-4-pyridinium ($MPP^+$), is a mitochondrial toxin that inhibits mitochondrial respiration and a dopamine

transporter substrate.[12–14] Therefore, MPTP degenerates dopamine neurons selectively. In drug screening for novel therapeutic agents for Parkinson's disease, this MPTP-induced Parkinson's model is often used, and the effects of various agents were judged by preservation of the striatal dopamine content.[6,8]

In the present study, we tried to establish a new drug screening method using imaging technique, and this time, we focused on a mouse model of Parkinson's disease using PPIS. To examine pre- and post-synaptic changes in dopamine neurons in the striata, we measured dopamine transporter availability and dopamine $D_1$ receptor binding with [$^{11}$C]$\beta$-CFT[17–20] and [$^{11}$C]SCH23390,[15,16] respectively. Furthermore, after PPIS measurements, we measured dopamine content in the striatum by HPLC and examined the correlation between it and [$^{11}$C]$\beta$-CFT or [$^{11}$C]SCH23390 binding *in vivo*.

## MATERIALS AND METHODS

*Animal preparation*

Studies were performed on 9-week-old 10 male C57BL/6NCrj mice purchased from Charles River Japan Inc. (Yokohama, Japan). All experiments were performed in accordance with the institutional guidelines of The Medical and Pharmacological Research Center Foundation and Central Research Laboratory, Hamamatsu Photonics. MPTP hydrochloride (Sigma-Aldrich Japan, Tokyo, Japan) was dissolved in saline, and saline or 20 mg/kg of MPTP was intraperitoneally administered 4 times a day at 2 h intervals. Seven days after saline or MPTP treatment, animals were anesthetized with an intraperitoneal administration of 1,500 mg/kg of urethane, fixed on an acrylic plate with thread and surgical tape (2 animals on each acrylic plate). Animals with an acrylic plate were placed at the center position between two PPIS detectors (the detector-detector distance was 30 cm), and two serial scans with labeled compounds were performed. After PPIS measurements, animals were sacrificed, and the striata were removed and stored at – 80°C until measurement of dopamine content.

*PPIS experiment*

Scans were performed with PPIS (IPS-1000-6XII; Hamamatsu Photonics, Hamamatsu, Japan).[5] This device consists of two opposing planar detectors, each having 4 columns × 6 rows detector units.[5] In the present study, the detector-detector distance became 30 cm, and mice heads were fixed at the center of detector units. In this condition, spatial resolution of this device becomes 1.6 mm FWHM in the focal plane.[5] To measure dopamine $D_1$ receptor binding and dopamine transporter availability, [$^{11}$C]SCH23390 (2 MBq) and [$^{11}$C]$\beta$-CFT (2 MBq) were intravenously administered to each animal, and dynamic image data were collected for 60 min (1 min × 60 time frames). In order to wait for a decrement of radioactivity of each tracer, more than 2 h-long intervals were placed between scans. Two regions of interest (ROIs) were set at the striatum and cerebellum, respectively, using the [$^{11}$C]SCH23390 images. These same ROIs were used for the analysis of [$^{11}$C]$\beta$-CFT images. Using the counts in the striatum and cerebellum, we defined specific binding in the striatum (SBS) as:

$$\text{SBS} = \frac{\text{counts in the striatum} - \text{counts in cerebellum}}{\text{counts in the cerebellum}}$$

In the above formula, to correct for differences in the injection dose of each tracer, counts in the striatum and cerebellum were used as the mean counts in the striatum and cerebellum from 40 min to 60 min after injection, because counts in the cerebellum 40 min after injection became almost constant (data not shown).

*Measurement of dopamine content in the striatum*

The striatum from each animal was homogenized with 1 m*l* of 0.1 M perchloric acid containing 0.1 mM ethylenediamine tetraacetic acid disodium using a glass-Teflon homogenizer, and stored for 30 min on ice. After centrifugation (16,000 *g*) for 15 min at 4°C, the supernatant was collected. Adding 0.1 M sodium acetate, the pH of supernatant was adjusted to about 3, and the dopamine content was measured by HPLC with electrochemical detection using isoproterenol as an internal standard.

*Statistical analysis*

Data are presented as the mean ± SD. All data were evaluated by analysis of variance (ANOVA) followed by Dunnett's multiple range test or unpaired t-test. $P < 0.05$ was considered significant.

## RESULTS

Seven days after saline or MPTP treatment, changes in the striatal [$^{11}$C]SCH23390 and [$^{11}$C]$\beta$-CFT binding are shown in Figure 2. The [$^{11}$C]SCH23390 binding was not changed, but the [$^{11}$C]$\beta$-CFT binding was significantly ($p < 0.05$) decreased by MPTP treatment.

In these animals, dopamine content in the striata measured by HPLC is shown in Figure 3. The MPTP treatment significantly ($p < 0.01$) decreased dopamine content (saline-treated: 11.7 ± 3.0 ng/mg tissue (n = 5); MPTP-treated: 2.3 ± 0.6 ng/mg tissue (n = 5)).

Correlation between striatal dopamine content and [$^{11}$C]SCH23390 or [$^{11}$C]$\beta$-CFT binding in the striatum is shown in Figure 4. The dopamine content in the striatum was significantly correlated with the binding of [$^{11}$C]$\beta$-CFT in the striatum ($r^2 = 0.625$, $p < 0.05$) (Fig. 4B), but not with that of [$^{11}$C]SCH23390 ($r^2 = 0.187$) (Fig. 4A).

## DISCUSSION

We examined the striatal dopamine $D_1$ receptor binding

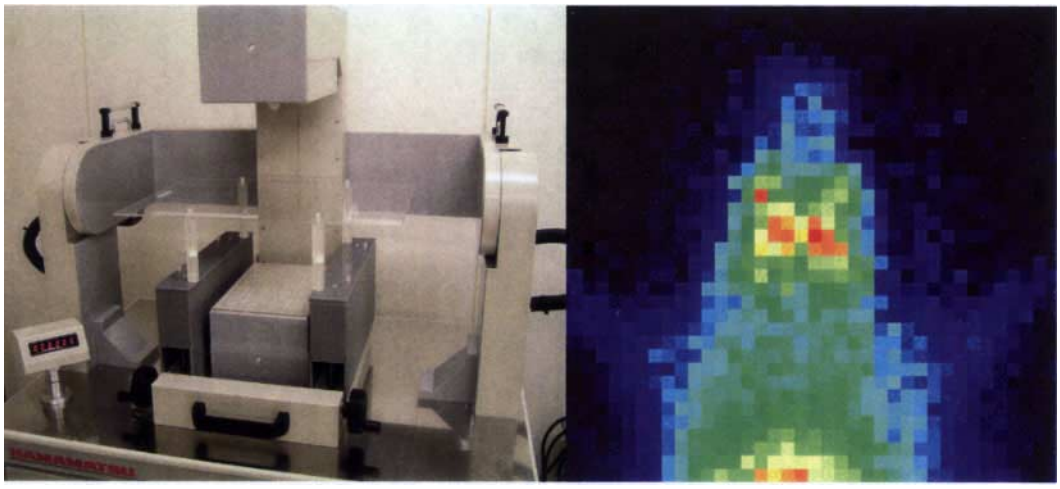

**Fig. 1** A picture of planar positron imaging system (*left*) and a typical brain image of [$^{11}$C]SCH23390 (*right*).

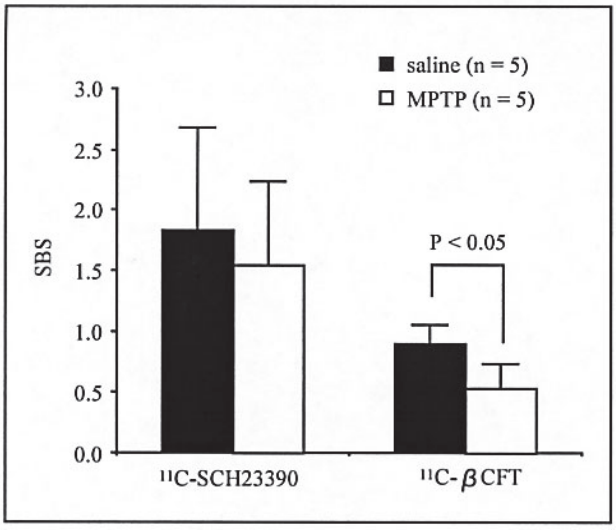


**Fig. 2** Mean binding of [$^{11}$C]SCH23390 and [$^{11}$C]$\beta$-CFT in the mouse striatum from 40 min to 60 min after injection. Closed and open columns indicate the saline- and MPTP-treated group, respectively. Each column represents the mean of 5 animals and the bar indicates S.D.

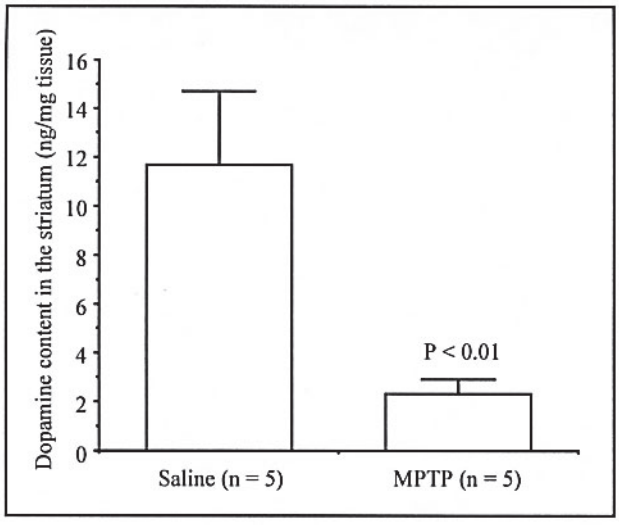


**Fig. 3** Dopamine contents measured by HPLC in the striatum 7 days after saline (n = 5) or MPTP (n = 5) treatment. Each column represents the mean and the bar indicates S.D.

and dopamine transporter availability using a novel planar positron imaging system (PPIS) with [$^{11}$C]SCH23390 and [$^{11}$C]$\beta$-CFT, respectively, seven days after MPTP treatment in mice. Dopamine transporter availability was significantly decreased by MPTP treatment. This observation is consistent with previous reports.[19–21] Dopamine content in the striatum was also significantly decreased in the MPTP group in the present study. This result has also commonly been reported in a mouse model of MPTP-induced Parkinson's disease.[6–8] Furthermore, dopamine content in the striatum significantly correlated with dopamine transporter availability in the striatum. These results reflect the fact that a metabolite of MPTP, $MPP^+$, is a substrate for the dopamine transporter and a mitochondrial toxin,[12–14] and MPTP treatment degenerated dopamine pre-synaptic neurons.

On the other hand, dopamine $D_1$ receptor binding in the striatum did not differ between the saline- and MPTP-treated groups. In humans, primates, and rodents with parkinsonism, the dopamine $D_1$ receptor in the striatum has been variously reported to be increased,[9,22] unchanged,[10,23–26] and decreased.[27,28] Therefore, we could not conclude from the present results that the $D_1$ receptor with parkinsonism was unchanged, and further time course studies may be required.

As shown above, to detect the changes in dopamine

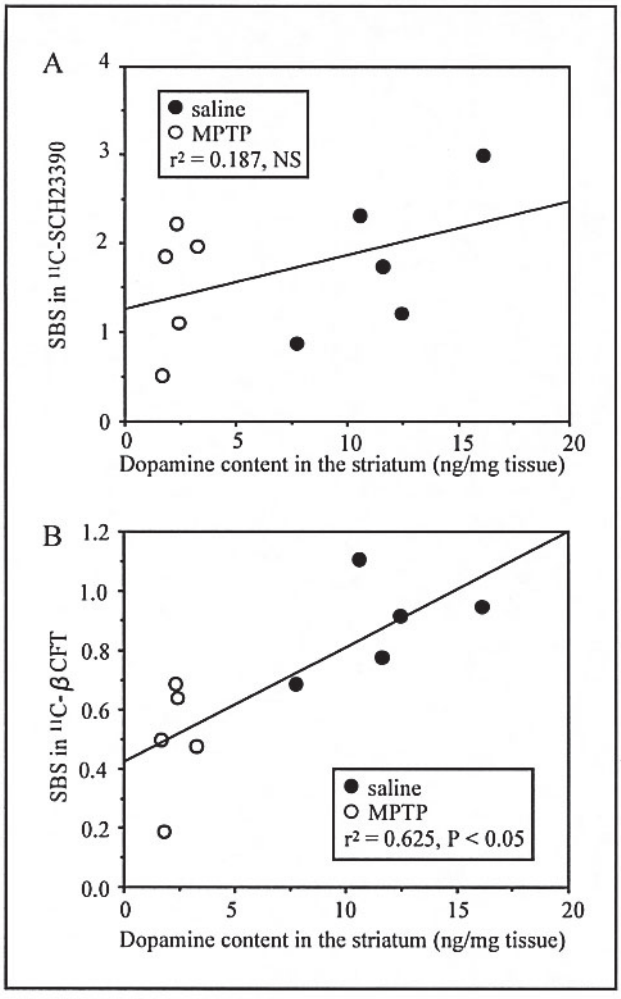


**Fig. 4** Correlations between dopamine content in the striatum and striatal binding for [$^{11}$C]SCH23390 (A) or [$^{11}$C]$\beta$-CFT (B) from 40 min to 60 min after injection. Seven days after saline or MPTP treatment, each PPIS scan and measurement of dopamine content were performed. Closed circles represent saline-treated animals and open circles MPTP-treated animals.

neurons in the MPTP-induced Parkinson's model using an imaging technique, it became clear that pre-synaptic imaging was useful and reflected biochemical changes in this model.

To develop novel therapeutic agents for Parkinson's disease, the MPTP-induced Parkinson's model is often used, and the effects of various agents were judged by preservation of the striatal dopamine content.[6,8] The present dopamine transporter availability measurement using PPIS may be an alternative screening method for anti-Parkinson's agents. In recent years, high throughput screening is required. Imaging technique, such as PET and this PPIS, may be able to reduce the number of animals necessary for one experiment. Furthermore, in the case of PPIS, the number of animals that could be measured at once depends on the area of the positron detectors, and this can increase with demand. Therefore, PPIS with [$^{11}$C]$\beta$-CFT may be a new convenient screening system for anti-parkinsonism agents. PPIS is a 2 dimension (projection) system, and therefore, reconstruction of images is not necessary, and good S/N data can be obtained using low radioactivity; indeed, we injected only 2 MBq of tracer per animal in the present study. The low level of radioactivity required in a scan can avoid saturation of receptor binding especially in small animal experiments, and also decrease the radiation risks to experimenters.

In order to utilize PPIS, distribution data of tracers are very important, because this system is a planar imaging system, and there is a possibility that accumulation of a tracer in other tissues located above and below of a target tissue might influence the accuracy of the data. In the present study, we confirmed them.

The present study demonstrated that PPIS could measure neurochemical and physiological functions conveniently in small animal experiments. This highlights the possibility of high throughput screening of new drugs using an imaging technique with PPIS.

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