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# Dopaminergic neuron destruction reduces hippocampal serotonin 1A receptor uptake of *trans*-[$^{18}$F]Mefway

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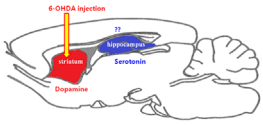
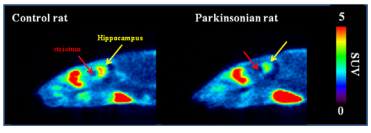
## HIGHLIGHTS

- The 5-HT system is implicated in mood related-non-motor symptoms of parkinson's disease.
- We examine changes of 5-HT$_{1A}$ receptor in a hemiparkinsonian rat model.
- The non-displaceable binding potential of HP and were calculated.
- The destruction of dopaminergic system causes the reduction of the serotonergic system.

## GRAPHICAL ABSTRACT

Serotonin PET after destruction of dopaminergic system.

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## ABSTRACT

The purpose of the present study is to investigate the relationship between dopaminergic neuron destruction and 5-HT system changes in a hemiparkinsonian rat model. We performed PET imaging studies with *trans*-[$^{18}$F]Mefway in a hemiparkinsonian model of unilateral 6-hydroxydopamine (6-OHDA) rats. Region-of-interests (ROIs) were drawn in the hippocampus (HP) and cerebellum (CB). HP uptake, the ratios of specific binding to non-specific binding in the HP, and non-displaceable binding potential ($BP_{ND}$) in the HP were compared between 6-OHDA and control rats. As a result, unilateral 6-OHDA-lesioned rats exhibited significant bilateral reduction of HP uptake and *trans*-[$^{18}$F]Mefway $BP_{ND}$ compared to the intact control group. Therefore, the results demonstrate that destruction of the dopaminergic system causes the reduction of the serotonergic system.



## 1. Introduction

Parkinson's disease (PD) is a slowly progressing disease and the second most common neurodegenerative disorder after Alzheimer's disease (Aarsland et al., 2012). Classically, PD is considered a motor

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disorder and is clinically diagnosed by the presence of at least two of the hallmark symptoms: resting tremor, bradykinesia, postural instability, and rigidity. The principal pathological characteristic of PD is the death of dopaminergic neurons in the nigrostriatal pathway, and motor symptoms become evident when 50–60% of dopaminergic neurons in the substantia nigra pars compacta (SNc) are lost (Tadaiesky et al., 2008). In addition, patients with PD often experience non-motor symptoms (NMS), such as depression, dementia, anxiety, fatigue, and sleep disorders. The previous study reported that these NMS are present even before the onset of motor symptoms, and they become more severe with disease progression (Barone, 2010). More than 60% of PD patients suffer from one or more NMS that impact their quality of life (Slaughter et al., 2001; Tadaiesky et al., 2008).

The serotonergic (5-HT) system is strongly implicated in mood related-NMS. Specifically, serotonin receptor subtype 1A (5-$HT_{1A}$), which is pre-and post-synaptically expressed in the midbrain and limbic system, is known to play a central role in maintaining stable 5-HT transmission (Popova and Naumenko, 2013). Post-mortem, pathological, and functional imaging studies have demonstrated 5-HT signaling dysfunction in PD. In post-mortem studies of patients with PD, 5-HT depletion was observed in both the caudate and frontal cortex (Fahn et al., 1971; Scatton et al., 1983). A pathological study revealed preferential loss of 5-HT in the caudate compared with the putamen but relatively low loss of 5-HT (66%) compared to dopamine (99%) (D'Amato et al., 1987; Kish et al., 2008; Scatton et al., 1983). in vivo imaging studies have also demonstrated the depletion of 5-HT innervations to the striatum as measured by decreased 5-HT transporter binding (D'Amato et al., 1987; Guttman et al., 2007).

From this perspective, the present study aimed to investigate the relationship between the dopaminergic neuron destruction and 5-HT system changes in a hemiparkinsonian rat model. We performed positron emission tomography (PET) imaging studies with *trans*-[$^{18}$F]Mefway, a 5-$HT_{1A}$ receptor-specific radioligand (Constantinescu et al., 2013; Wooten et al., 2011), in a hemiparkinsonian model of unilateral 6-hydroxydopamine (6-OHDA) rats (Blandini et al., 2008; Duty and Jenner, 2011).

## 2. Materials and methods

### 2.1. Animals and lesioning

The study employed seven male Sprague–Dawley (SD) rats weighing 280–320 g (Orient Bio, Inc., Seongnam, Korea) unilaterally lesioned with 6-hydroxy dopamine (6-OHDA; Sigma-Aldrich, St. Louis, MO, USA). Lesioning was performed as follows. Rats were pretreated with desipramine hydrochloride (12.5 mg/kg; intraperitoneal [i.p.] injection, 12.5 mg/ml, 0.1 ml/100 g) before anesthesia. Surgery was performed under deep intraperitoneal ketamine/xylazine anesthesia (doses of 40 mg/kg and 5 mg/kg, respectively). Each animal's head was shaved and placed in a stereotaxic device (Stoelting Co., Wood Dale, IL, USA). According to the rat brain atlas (Paxinos and Watson, 2007), 6-OHDA was injected into two sites in the right striatum (20 μg/4 μl/site, total of 40 μg 6-OHDA); the targets (relative to the bregma and dura) were anterior–posterior (AP) +0.5 mm, medial–lateral (ML) 2.5 mm, dorsal–ventral (DV) −5.0 mm and AP −0.5 mm, ML 4.2 mm, DV −5.0 mm at a rate of 1 μl/min using a 26G Hamilton syringe. The inserted needle was withdrawn from each location after 5 min, and the wound was sutured. The rats were allowed 5 weeks recovery before undergoing PET scanning. Throughout the study, animals were housed in a temperature- and humidity-controlled room with a 12-h light/dark cycle and free access to food and water. The exact same procedure was done for the control rats ($n=5$, weighing 284–315 g), except that normal saline was administered to the striatum. These experimental protocols were approved by the committee for the care and use of laboratory animals of Yonsei University College of Medicine.

### 2.2. Assessment of rotational behavior

Rotational behavior was tested using a multichannel rotometer system (ROTORAT, MED Associates, Inc., St. Albans, VT, USA). Contra- or ipsilateral rotational behaviors were induced by i.p. injection of D-amphetamine (5 mg/kg) 14 days after 6-OHDA injection. Each animal was placed in a cylindrical test chamber for 60 min, and counter-clockwise rotations were analyzed. Animals showing more than 100 rotations/min were considered successful and used for further experiments. There were five rats in total.

### 2.3. Radioligand

Radiosynthesis of *N*-[2-{4-(2-methoxyphenyl)-piperazinyl}ethyl]-*N*-(2-pyridyl)-*N*-(4-*trans* [$^{18}$F]fluoromethylcyclohexanecarboxamide) was performed according to methods reported in the previous studies (Choi et al., 2013; Saigal et al., 2013).

### 2.4. Inhibition of skull uptake

We previously confirmed that the antifungal agent fluconazole effectively inhibits defluorination of *trans*-[$^{18}$F]Mefway and can effectively reduce spillover of radioactivity from the skull (Choi et al., 2012). For this reason, fluconazole (60 mg/kg, OneFlu injection, JW Pharmaceutical, Seoul, Korea) was pre-administered through the tail vein to the anesthetized rat with an infusion pump (Harvard pump 11 plus, Harvard Apparatus,USA) for 1 h.

### 2.5. PET studies

After pre-administration of fluconazole, anesthetized rats were placed in the center of a gantry of a PET scanner. Emission data collection was started at the time of *trans*-[$^{18}$F]Mefway injection, and radiotracer accumulation in the brain was examined by dynamic PET scans over 120 min after injection of 15–20 MBq *trans*-[$^{18}$F]Mefway. After emission, attenuation corrections were performed using data from a 10-min transmission scan with a $^{57}$Co point source.

PET scanning was undertaken with a Siemens Inveon small animal PET scanner (Siemens Medical Solutions, Malvern, PA, USA). The scanner has a peak absolute system sensitivity of $\geq$10% in the 250–750 keV energy window, an axial field of view of 12.7 cm, 1.4 mm full width center of field of view at half maximum (FWHM) spatial resolution and a transaxial field of view of 10 cm.

### 2.6. Image analysis

Raw list mode data were reconstructed in user-defined time frames (10 s × 6 frames, 30 s × 8 frames, 300 s × 5 frames, 1800 s × 3 frames) with voxel dimensions of 0.86 × 0.86 × 0.80 $mm^3$ by the 2-dimensional order-subset expectation maximization (OSEM) algorithm. Region-of-interests (ROIs) analysis via AsiProVM (Acquisition Sinogram Image Processing software, CTI Molecular Imaging, Knoxville, TN, USA) was used to evaluate the acquired image files. ROIs were drawn in the hippocampus (HP) and cerebellum (CB), and time–activity curves of the *trans*-[$^{18}$F]Mefway in these regions were acquired.

The CB ROI was used to estimate skull uptake and was chosen as a reference region; the signal of this area was regarded as non-specific because adult rat CB contains few 5-$HT_{1A}$ receptors.