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VOXEL-BASED MORPHOMETRY OF THE MARMOSET BRAIN: *IN VIVO* DETECTION OF VOLUME LOSS IN THE SUBSTANTIA NIGRA OF THE MPTP-TREATED PARKINSON'S DISEASE MODEL

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Abstract—Movement dysfunction in Parkinson's disease (PD) is caused by the degeneration of dopaminergic (DA) neurons in the substantia nigra (SN). Here, we established a method for voxel-based morphometry (VBM) and automatic tissue segmentation of the marmoset monkey brain using a 7-T animal scanner and applied the method to assess DA degeneration in a PD model, 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP)-treated animals, with tyrosine-hydroxylase staining. The most significant decreases of local tissue volume were detected in the bilateral SN of MPTP-treated marmoset brains (−53.0% in right and −46.5% in left) and corresponded with the location of DA neurodegeneration found in histology (−65.4% in right). In addition to the SN, the decreases were also confirmed in the locus coeruleus, and lateral hypothalamus. VBM using 7-T MRI was effective in detecting volume loss in the SN of the PD-model marmoset. This study provides a potential basis for the application of VBM with ultra-high field MRI in the clinical diagnosis of PD. The developed method may also offer value in automatic whole-brain evaluation of structural changes for the marmoset monkey.

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Abbreviations: Cd, caudate nucleus; DA, dopaminergic; GM, gray matter; LC, locus coeruleus; LH, lateral hypothalamus; MPTP, 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine; MRI, magnetic resonance imaging; PBS, phosphate-buffered saline; PD, Parkinson's disease; Pu, putamen; ROI, region of interest; SN, substantia nigra; SNC, substantia nigra pars compacta; T1WI, T1-weighted imaging; TH, Tyrosine-hydroxylase; VBM, voxel-based morphometry; WM, white matter.

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INTRODUCTION

Parkinson's disease (PD) is a progressive movement disorder caused by the degeneration of dopaminergic (DA) neurons in the substantia nigra (SN) (Jankovic, 2008). Symptoms of PD appear when the loss of DA neurons from the SN exceeds 50–70% of the normal control (Marsden, 1990; Damier et al., 1999) and were also found to accompany reduction of SN width, detected using magnetic resonance imaging (MRI) (Pujol et al., 1992).

Voxel-based morphometry (VBM) (Ashburner and Friston, 2000) is a method which allows for the evaluation of local tissue volume changes over the entire brain, independent of operator or region of interest (ROI), and is used for the evaluation of brain atrophy in various neurological and psychological disorders (Mueller et al., 2012a,b). Therefore, VBM may also have a potential utility in evaluating volume loss in the SN due to degeneration of DA neurons. Several studies used VBM to detect volume decreases in brain areas of PD patients, including the brain stem, striatum, limbic system, and cortex (Burton et al., 2004; Camicioli et al., 2009; Jubault et al., 2009; Wattendorf et al., 2009). However, to our knowledge, there have been no reports on the detection of SN volume loss in PD patients using VBM. Moreover, the causal relationship between MRI volume reductions in the nigrostriatal system and degeneration of DA neurons in the SN remains unclear.

For a preclinical MRI study of PD, the common marmoset (*Callithrix jacchus*), a small New World primate species, is a suitable animal model to investigate whether VBM can detect volume loss in the nigrostriatal system due to loss of DA neurons in the SN. A PD-model marmoset has been established through administration of 1-methyl-4-phenyl-1,2,3,6-tetra hydropyridine (MPTP) (Gerlach and Riederer, 1996; Ando et al., 2008, 2012, 2014), which exhibits selective DA neurodegeneration in the SN and PD-like signs including movement tremors, immobility, muscle rigidity, and positional dysfunction (Ando et al., 2008). The marmoset also shares similar brain anatomy to that of humans, the existence of neuromelanin in DA neurons and an internal capsule separating the striatum into the caudate nucleus (Cd) and putamen (Pu).

The purposes of this study were to establish an automatic VBM method for the marmoset brain and apply the method in detecting SN volume loss due to DA neurodegeneration in the MPTP-treated non-human primate PD model.

EXPERIMENTAL PROCEDURES

Monkeys

Eight male marmosets (*C. jacchus*; CLEA Japan, Tokyo, Japan) were used for the present study. All procedures were performed in accordance with the Laboratory Animal Welfare Act and the Guide for the Care and Use of Laboratory Animals (National Institutes of Health, USA). The protocol of the present study was reviewed by the Institutional Animal Care and Use Committee and approved by the Central Institute for Experimental Animals (CIEA) of Japan (CIEA Approval no. 09025A). Six marmosets (age, 2.9 ± 0.6 years; body weight, 326.8 ± 27.1 g) received subcutaneous administration of MPTP on three consecutive days at daily doses of 2, 2, and 1 mg/kg. This procedure results in long-lasting and stable PD-like signs, such as tremor and immobility, measured objectively as decreased locomotion count (Ando et al., 2008, 2012). The other two marmosets were used as intact controls for histological evaluation.

MRI

All MRI was performed using a 7-T Biospec 70/16 MRI system (Bruker Biospec GmbH; Ettlingen, Germany). The inner diameter of the integrated transmitting and receiving coils was 62 mm.

T1-weighted imaging (T1WI)

For longitudinal evaluation of the distribution affected by degenerated DA neurons, T1WI was performed before and 10 weeks after MPTP administration. Before the MRI, the marmosets were deeply anesthetized with an intramuscularly administered mixture of 0.25-ml ketamine (50 mg/kg; Ketalar; Daiichi Sankyo, Tokyo, Japan) and 0.04-ml xylazine (5 mg/kg; Selactar; Bayer, Leverkusen, Germany). The combined dose included 0.6 ml/kg of ketamine and 0.1 ml/kg of xylazine. Once anesthetized, the marmosets were administered a mixture of oxygen and 2.5% isoflurane (Abbott Laboratories, Abbott Park, IL, USA) at a constant rate through a tracheal intubation and artificial respirator (SN-480-7; Shinano, Tokyo, Japan). To reduce motion artifacts caused by blood and cerebrospinal fluid, the marmosets were immobilized on an acrylic bed with a specially designed head positioner, and an electrocardiogram probe (SA Instruments, Stony Brook, NY, USA) was attached to the marmoset's ventral thorax (Yamada et al., 2008).

High-resolution T1WI data were acquired using an optimized magnetization-prepared rapidly acquired gradient-echo sequence for marmoset brain with 7-tesla (Hikishima et al., 2011). The imaging parameters were as follows: repetition time/echo time, 13/3.8 ms; inversion time, 1500 ms; time to delay, 3700 ms; 51.2 × 51.2 × 25.6-mm field of view on a 256 × 256 × 128 matrix; number of segments, 4; and number of averages, 2. The T1WI scan required 1 h 40 min.

Histological analysis

To confirm the distribution and amount of volume loss in the brain using VBM, we performed histological analysis of DA neurons after MRI. Intact and MPTP-treated marmosets ($n = 2$ each) were perfused intracardially with 4% paraformaldehyde/phosphate-buffered saline (PBS) and then paraformaldehyde in PBS (4% PFA). The brains were removed and post-fixed in 4% PFA. The brains were embedded in paraffin and prepared into 240 serial sagittal sections at 15-μm thickness. Immunohistochemical staining with a Tyrosine-hydroxylase (TH) antibody (1:500, mouse anti-TH; Millipore #MAB318; Bilerica, MA, USA) was performed using an automated immunostainer (Bond-MAX; Leica Microsystems, Wetzlar, Germany). Sections were then counterstained with hematoxylin.

Additionally TH and Klüver–Barrera staining of the coronal sections at 20-μm thickness in the intact and MPTP-treated brain were performed. All slides were scanned with a slide scanner (SCN400; Leica Microsystems, Wetzlar, Germany).

In addition to visual inspection of DA neurons, we counted the number in the SN from three sagittal sections spaced 30 μm (lateral 2.6 mm) apart and measured the volume of TH-positive cluster in the SN from 3D histology data of intact and MPTP-treated marmoset brains. The detail of the creation of 3D histology data is described in our previous study (Hikishima et al., 2015).

Voxel-based morphometry

Automatic VBM was performed in the following steps (Fig. 1):

(1) 3D T1WI

All T1WIs were obtained using the method described above. Since the marmoset brain (approximately 8 cm^3) is far smaller than the human brain (approximately 1400 cm^3), voxel size of marmoset MRI were first multiplied by five to make the images usable in software for human brains ($3\sqrt{(1400\ cm^3/8\ cm^3} = 5.59$).

(2) Non-uniformity correction

To improve brain segmentation accuracy, the uniformity of all images were corrected using N3 software (http://www.bic.mni.mcgill.ca/software/N3/) (Sled et al., 1998), which was also adapted for small animal 7-T MRI (Lin et al., 2013).

(3) Denoising

All images were denoised using a 3D non-local mean (NLM) filter implemented in Amira version 5.4 (FEI Visualization Sciences Group, Burlington, MA, USA), which can remove noise while preserving edges (Coupe et al., 2008).