Characterizing the Modulation of mGluR5 in a 6-OHDA-induced Rat Model of Parkinson's Disease

by

Peter Lamb

Submitted to the Department of Nuclear Science and Engineering in Partial Fulfillment of the Requirements for the Degree of

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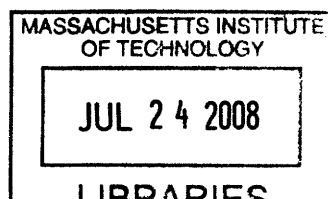
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ABSTRACT

MicroPET imaging studies were conducted to investigate the role of metabotropic glutamate subtype-5 receptors (mGluR5) in Parkinson's disease (PD). Four analogical PET ligands were used to characterize modulation of mGluR5 function in a 6-hydroxydopamine (6-OHDA) induced rat model of PD. Unilateral 6-OHDA lesions were made in the right medial forebrain bundle, and severity of these lesions was determined with $[^{11}C]$CFT. The binding characteristics of the PET ligands were analyzed using a modified distribution volume method of the Logan reference region model. Binding potential values were calculated on the striatum, hippocampus, and cortex, using the cerebellum as a reference tissue.

On the right (with lesion) side of the striatum, $[^{11}C]$CFT binding decreased. Three of the four investigated mGluR5 ligands ($[^{11}C]$MPEP, $[^{11}C]$M-PEPy, and $[^{11}C]$M-MPEP) also showed enhanced binding characteristics on the same side of the brain. The right hippocampus and cortex showed similar results. The mGluR5s' enhanced binding characteristics on the right side of the brain suggest a complementary and compensatory role of metabotropic glutamate receptors in the dopaminergic neurodegeneration of Parkinson's disease.

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1. Introduction

Parkinson's disease is a progressive, neurodegenerative disorder of the central nervous system that specifically affects the motor system. Movement control is regulated by the direct and indirect pathways of the basal ganglia (Rouse et al., 428). Research has shown that Parkinson's disease is a result of the degeneration of dopamine-producing neurons in the substantia nigra, which is part of the basal ganglia (Alexi et. al., 411). The loss of dopamine, a neurotransmitter that communicates signals within the brain, affects the direct pathway and leaves those affected unable to control their muscle movements. The exact cause of the loss of dopamine is unknown and much Parkinson's disease research has focused on this anomaly.

The glutamatergic neurotransmitter system is closely linked with the dopaminergic neurotransmitter system. Glutamate is the main excitatory neurotransmitter of the central nervous system and is part of the indirect pathway of muscle control (Rouse et al., 428). The balance between direct and indirect pathways is regulated by dopaminergic neurons in such a way that dopamine has an activating effect on the direct pathway and a deactivating effect on the indirect pathway (Rouse et al., 428). Dopaminergic cell death associated with Parkinson's disease causes a decrease in the direct pathway of motor control. In order to compensate for this loss of function, there is activation and increase of the indirect pathway (Dekundy et. al, 318). Under normal neural conditions when there are few neuronal transmissions through the indirect pathway, glutamate levels in the basal ganglia are kept low. However, under Parkinsonism conditions, glutamate concentration increases to such an extent that it

becomes an excitotoxin[1], contributing to neuronal cell death of the basal ganglia associated with Parkinson's disease. Stopping or decreasing this rise in glutamate is theorized to help alleviate the symptoms of Parkinson's disease.

When neurotransmitters such as glutamate fire signals within the brain, the signals are picked up by neurons' receptors. Binding a glutamate antagonist to these receptors would necessarily prevent the transmission of glutamate. There are two main classes of receptors: ionotropic and metabotropic. Glutamate antagonists of the ionotropic receptors, such as N-methyl-D-aspartate (NMDA), have been shown to block the binding of glutamate to neuron receptors and produce antiparkinsonian effects (Dekundy et. al, 318). However, these receptor antagonists also produce psychotomimetic symptoms and ataxia, which limit their potential for clinical use (Dekundy et. al, 318). Therefore, researchers have turned to investigating antagonistic metabotropic glutamate receptors[2] (mGluRs). Group I receptors, specifically mGluR5[3], have been implicated in the pathogenesis of Parkinson's disease (Dekundy et. al, 318). Therefore, inhibition of mGluR5 has been proposed as a therapeutic treatment of Parkinson's disease.

We sought to further investigate the role of mGluR5 in Parkinson's disease. Specifically, four analogical PET ligands[4] were used to characterize modulation of

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[1] Neurodegeneration is caused by three neuronal cell mechanisms: metabolic compromise, excitotoxicity, and oxidative stress (Alexi et al., 409). Each of these three mechanisms is believed to play a role in causing Parkinson's disease.

[2] There are eight mGluR subtypes which are divided into three groups: Group I (mGluR1 and mGluR5), Group II (mGluR2 and mGluR3), and Group III (mGluR4, mGluR6, mGluR7, and mGluR8). (Dekundy et al., 318).

[3] As evidenced by the research of Awad et al. (Dekundy et. al, 318).

[4] 2-[^{11}C]methyl-6-(2-phenylethynyl)pyridine ([^{11}C]MPEP), 2-(2-(3-[^{11}C]methoxyphenyl) ethynyl) pyridine ([^{11}C]M-MPEP), 2-(2-(5-[^{11}C]methoxypyridin-3-yl)ethynyl) pyridine ([^{11}C]M-PEPy), and 3-[(2-[^{18}F]methyl-1,3-thiazol-4-yl)ethynyl]pyridine ([^{18}F]M-TEP).

mGluR5 function in a 6-OHDA[5] induced rat model of Parkinson's disease. 45 PET

imaging studies were performed 4-6 weeks after unilateral injection of 6-OHDA into the

right medial forebrain bundle. Severity of the lesion was determined with [^{11}C]CFT

([^{11}C]2-β- carbomethoxy-3-β-(4-fluorophenyl)tropane), a specific and sensitive ligand for

imaging dopamine transporter function (Personal communication, Aijun Zhu, PhD). The

right (with lesion) side of the brain showed enhanced mGluR5 binding characteristics,

suggesting a complementary and compensatory role of metabotropic glutamate receptors

in the dopaminergic neurodegeneration of Parkinson's disease.

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[5] 6-OHDA is a selective catecholaminergic neurotoxin and is structurally similar to dopamine. The compound is taken up and concentrated in dopamine cells, resulting in degeneration of dopaminergic neurons. (Personal communication, Aijun Zhu, PhD).

2. Methods

2.1 The 6-OHDA Rat Model

In order to investigate the role of mGluR5 in Parkinson's disease, we used the unilateral lesion, 6-hydroxydopamine (6-OHDA) rat model. There are various Parkinson's disease models for rats that use 6-OHDA. One advantage of using a unilateral lesion is that the loss of dopaminergic neurons in the lesion side can be investigated and compared to the intact part on the contralateral side (Personal communication, Aijun Zhu, PhD).

Nine male Sprague-Dawley rats, weighing 300-350 g, were used for the PET studies. Dopaminergic system failure was triggered by unilateral injection of 6-OHDA (8μg, 2μ - Sigma-RBI) into the medial forebrain bundle on the right side of the brain (coordinates: AP −4.5; ML +1.2; DV −7.6) using a Hamilton syringe. In preparation of injection, rats were anesthetized with ketamine[6] (80mg/kg i.p.) and xylazine[7] (10mg/kg i.p.), and then placed into the Kopf stereotaxic frame. Thirty minutes prior to the 6-OHDA injection rats received desipramine[8] (20mg/kg i.p.) to restrict the effect of 6-OHDA to the dopaminergic neurons. Experiments were performed in accordance with the National Institute of Health Guide for the Care and Use of Laboratory Animals and approved by the Institutional Policy by the Subcommittee on Research Animals of the

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[6] Ketamine is a dissociative anesthetic. This group of anesthetics is a cyclohexamine compound that uncouples sensory, motor, integrative, memory, and emotional activities in the brain (Personal communication, Daniela Pellegrino, PhD).
[7] Xylazine is a thiazine derivative and an alpha-2 adrenergic agonist. This compound causes central nervous system depression resulting in sedation, emesis, and mild analgesia. Xylazine is very useful for anesthesia in rodents, especially when combined with other drug, such as Ketamine (Personal communication, Daniela Pellegrino, PhD).
[8] Desipramine is a noradrenalin uptake blocker that restricts the damage induced by 6-OHDA to the dopaminergic system (Personal communication, Daniela Pellegrino, PhD).

Harvard Medical School and Massachusetts General Hospital (Personal communication, Aijun Zhu, PhD).

2.2 Radioligands[9]

Severity of the 6-OHDA lesion was determined with 2-β-carbomethoxy-3-β-(4-fluorophenyl)tropane ([11C]CFT), a specific and sensitive ligand for imaging dopamine transporter function. Four analogical PET ligands were used to characterize modulation of mGluR5 in our rat model: 2-[11C]methyl-6-(2-phenylethynyl)pyridine ([11C]MPEP), 2-(2-(3-[11C]methoxyphenyl)ethynyl)pyridine([11C]M-MPEP), 2-(2-(5-[11C] methoxy pyridin-3-yl)ethynyl)pyridine ([11C]M-PEPy), and 3-[(2-[18F]methyl-1,3-thiazol-4-yl)ethynyl]pyridine ([18F]MTEP).

Radiosynthesis[10] of [^{11}C]MPEP, [^{11}C]M-MPEP, and [^{11}C]M-PEPy was done according to Yu et al (Yu et al, 1999/2005). For the radiosynthesis of [^{18}F]MTEP, the precursor 2-bromo-5-(2-(2-methylthiazol-4-yl)ethynyl)pyridine (~2.0 mg) was added to the dried [18F]fluoride/Kryptofix 2.2.2 vial with dry DMSO (0.5 ml). The vial was heated at 100°C for 10 minutes. After cooling for 5 minutes, a sample was taken to the radio TLC, showing that 72% of the reactivity was in the desired product, 2-[^{18}F]fluoro-5-(2-(2-methylthiazol-4-yl)ethynyl)-pyridine, i.e. [^{18}F]MTEP, and the product was identified with an authentic reference standard. The reaction mixture was diluted with 3

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[9] Ligands are biological compounds used to study metabolic and physiologic functioning and pathways. PET chemists attach radioactive, positron-emitting isotopes to ligands, thus making radioligands. PET scanners detect positron emission from these radioligands and use this data to generate cross-sectional images that show area of activity of the radioligands. This information can be used to characterize modulation of radioactivity from the radioligand as a function of position in the area being imaged (microPET® Operating Instructions. (2002). Concorde Microsystems, Inc., Knoxville).

[10] Radiosynthesis follows standard lab protocol (Personal communication, Aijun Zhu, PhD).

ml EtOAc, loaded to a silica Sep-Pak, and eluted with 5 ml more EtOAc. The EtOAc

solution was evaporated at 130°C under a N_2 flow. The residue (~0.5 ml) was cooled for

5 minutes, diluted with HPLC solvent (1.5 ml), and transferred to an HPLC injection vial.

By using a μ-Bondapak column and a mobile phase of 40% methanol and 60% phosphate

buffer (pH 7.4, 0.15 mM), the pure [^{18}F]MTEP was isolated. Radiosynthesis of [^{11}CFT]

was done according to Brownell et al (Brownell et al, 1996).

2.3 PET Imaging

PET Imaging was performed using the microPET® scanner designed by Concorde

Microsystems, Inc. Imaging was conducted 2 weeks after injection of 6-OHDA and

again at 4 weeks to accurately determine dopaminergic deficit. At this point there was a

sufficient decrease in dopaminergic levels to begin the mGluR5 experiments, which

continued for the last two weeks of the study (weeks 4 to 6).

Individual rats were securely fixed in a stereotaxic head holder equipped with a

gas inhalation system and anaesthetized by gas (1-1.5% isoflurane, 2% nitrous oxide and

oxygen flow of 1 L/min). The tail vein was then catheterized for administration of a

radiolabeled mGluR5 ligand (500uCi ~ 1300uCi). The rat was then placed ventrally into

imaging position at the center of the imaging field. A heated waterbed and an infrared

light maintained rat body temperature while the HESKA Vet/Ot Plus 4800 monitored

heart rate throughout the duration of the imaging process.

Transmission imaging was conducted using a rotating germanium-68 (Ge-68) pin source to account for attenuation correction[11]. The radiolabeled ligand was injected into the tail vein and volumetric dynamic data was acquired by the scanner in list-mode format for 60 min. [^{11}C]CFT studies were used to investigate dopamine transporter function and were conducted twice in each animal. The mGluR5 function was investigated with: [^{11}C]MPEP in 2 rats, [^{11}C]M-MPEP in 6 rats, [^{11}C]M-MPEPy in 7 rats, and [^{18}F]MTEP in 3 rats.

2.4 Image Analysis

Image analysis was performed using ASIPro^{TM} version 6.0, Concorde Microsystem's Acquisition and Analysis Tool. Dynamic PET imaging data was corrected for uniformity, attenuation and decay factors, and processed by filtered back projection (Personal communication, Aijun Zhu, PhD). A modified distribution volume method of the Logan reference region model[12] was used to analyze the PET ligand binding characteristics. The Logan linear model was used to determine the distribution

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[11] Positrons are positively charged counterparts to electrons. After emission from an isotope, positrons will collide with electrons and annihilate each other. This annihilation releases energy in the form of two simultaneous 511 keV gamma rays, which are emitted at almost 180° from each other. The PET scanner is able to detect these rays, and this is known as a coincidence event. Coincidence events are transferred back to a host computer that uses this information to generate cross-sectional images that delineate areas of activity in the radiolabeled compound. (microPET® Operating Instructions. (2002). Concorde Microsystems, Inc., Knoxville.) However, coincidence events must be corrected for random coincidences, photon scattering, etc. Transmission imaging and attenuation correction is used in the correction process so that an accurate image of activity can be reconstructed.

[12] The Logan model is a data analysis method of PET imaging. This particular graphically derived reference region model uses a reference region, the cerebellum, as the region free of specific radioligand binding. By plotting the data according to $\frac{\Sigma_0^T C_t(t)\Delta t}{C_t(T)} = DVR \times \frac{\Sigma_0^T C_r(t)\Delta t}{C_t(T)} + C'$, where $C_t(t)$ and $C_r(t)$ are radioligand concentrations from the region of interest and reference region, respectively, a Logan plot is obtained by plotting $\Sigma_0^T C_t(t)\Delta t$ vs. $\Sigma_0^T C_r(t)\Delta t$, which yields a direct measure of the distribution volume ratio (DVR) of the radioligand (the slope of this plot). (Christian et. al., 73). The DVR is the ratio of the radioligand concentration in tissue to that in plasma at equilibrium. Therefore, a DV of 15 means that there is a radioligand concentration in tissue of 15:1 (Turku PET Centre). From the DVR the binding potential of the radioligand can be calculated.

volume (DV) of ligands, which can be calculated from the regions of interest (striatum, hippocampus, and cortex) and the receptor-free reference area (cerebellum). We graphically obtained the distribution volume ratio (DVR) in order to calculate binding potentials of the ligands. Binding potential values were obtained using the equation BP=DVR-1 (Personal communication, Aijun Zhu, PhD).

Two methods were applied for drawing the regions of interest (ROIs) on the PET images: studies conducted with an mGluR5 ligand and [^{11}C]CFT during the same imaging session or separate imaging sessions. ROIs were drawn and loaded directly to the PET images of the mGluR5 ligand if the studies were conducted with an mGluR5 ligand and [^{11}C]CFT ligand during the same session. This method allows analyses and accurate local comparison of the mGluR5 and dopamine transporter data of the same brain regions. If ligand and CFT studies were conducted during separate sessions, mGluR5 images were fused to [^{11}C]CFT images on the same rat. This merging procedure was done manually on a pixel-by-pixel basis using the Harderian glands, olfactory bulb, and the whole brain contours as internal markers (Personal communication, Aijun Zhu, PhD). ROIs of the right and left striatum, cortex, hippocampus, and cerebellum were then drawn on the [^{11}C]CFT images and applied to the PET images of the mGluR5 ligands. From these ROIs, time activity curves (TACs) were generated and percent differences of the right-left regions were calculated[13]. The percent difference (Φ) of the DVRs of [^{11}C]CFT binding in the striatum is a measure of the deficit in the dopamine transporter function as, as well as a measure of the severity of damage on a presynaptic dopamine terminal (Personal communication, Aijun Zhu, PhD).

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[13] Percent differences of the right-left regions were calculated by the following formula:
$\Phi = \dfrac{DVR_{right} - DVR_{left}}{DVR_{left}} \times 100$, where Φ is the percent difference (Personal communication, Aijun Zhu, PhD).

The percent differences (Φ) of the DVRs of [11C]MPEP, [11C]M-MPEP, [11C]M-MPEPy, and [18F]MTEP binding are measures of the modulation of mGluR5 ligands in the striatum of PD-model rats (Personal communication, Aijun Zhu, PhD).

3. Results

3.1 PET Images

Dynamic PET images showing activity distribution of the dopamine transporter ligand [^{11}C]CFT and the four mGluR5 ligands [^{11}C]MPEP, [^{11}C]M-MPEP, [^{11}C]M-PEPy, and [^{18}F]MTEP are presented in Figure 1. Since [^{11}C]CFT is a dopamine transporter ligand, areas which have high [^{11}C]CFT accumulation (and thus have high dopamine concentration), will appear red (high concentration) on the image. 6-OHDA, a dopamine neurotoxin, was injected into the right side of the brain. Therefore, dopaminergic regions of the right side of the brain, such as the striatum and hippocampus, should have low [^{11}C]CFT accumulation and will appear yellow (medium concentration) on the image. In these same areas, glutamate levels should have increased. Thus, mGluR5 ligands should be red (high accumulation) in these two regions.

As Figure 1 shows, the left striatum and hippocampus have high [^{11}C]CFT accumulation. Contrastingly, mGluR5 ligands have high accumulation in the right striatum and hippocampus.
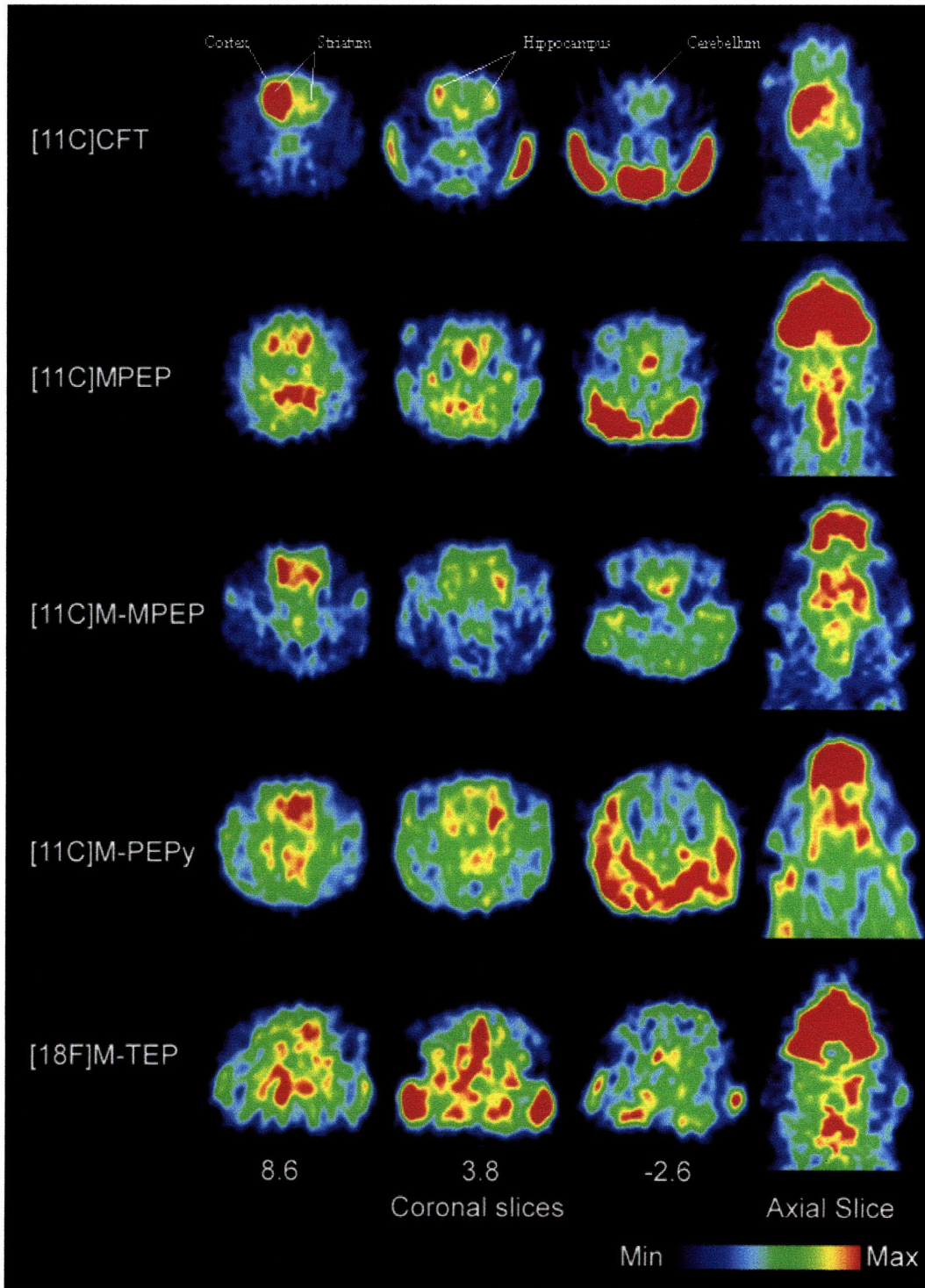
Figure 1. **Accumulation of [^{11}C]CFT, [^{11}C]MPEP, [^{11}C]M-MPEP, [^{11}C]M-PEPy, and [^{18}F]MTEP in the 6-OHDA lesioned rat brain.**

Activity distribution after administration of mGluR5 ligands are illustrated at 7-10 minutes and [^{11}C]CFT at 25-40 minutes. The first column shows coronal images at the striatum level, the second column at the hippocampus level, the third column at the cerebellum level, and the fourth column (axial slice) at the mid-striatal level.

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3.2 Time Activity Curves

Time activity curves were generated from the striatum, hippocampus, and cerebellum and are reproduced in Figure 2A. The corresponding Logan plots[14] of these curves are shown in Figure 2B. As mentioned in the Image Analysis section of the Methods, time activity curves (TACs) were generated from the regions of interest and corresponding Logan plots were used to calculate the DVR of radioloigand binding in these regions of the brain. The percent differences of DVRs of [^{11}C]MPEP, [^{11}C]M-MPEP, [^{11}C]M-MPEPy, and [^{18}F]MTEP binding are measures of the modulation of mGluR5 ligands in the striatum of PD-model rats. (Personal communication, Aijun Zhu, PhD).

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[14] The Logan plots (straight lines) were obtained using the equation: $\frac{\sum_0^T C_t(t)\Delta t}{C_t(T)} = DVR \times \frac{\sum_0^T C_r(t)\Delta t}{C_t(T)} + C'$,

where T=10 min. (Christian et. al., 73). For all compounds, the Logan plots show linearity starting at 10 min after injection of the ligand (Personal communication, Aijun Zhu, PhD).
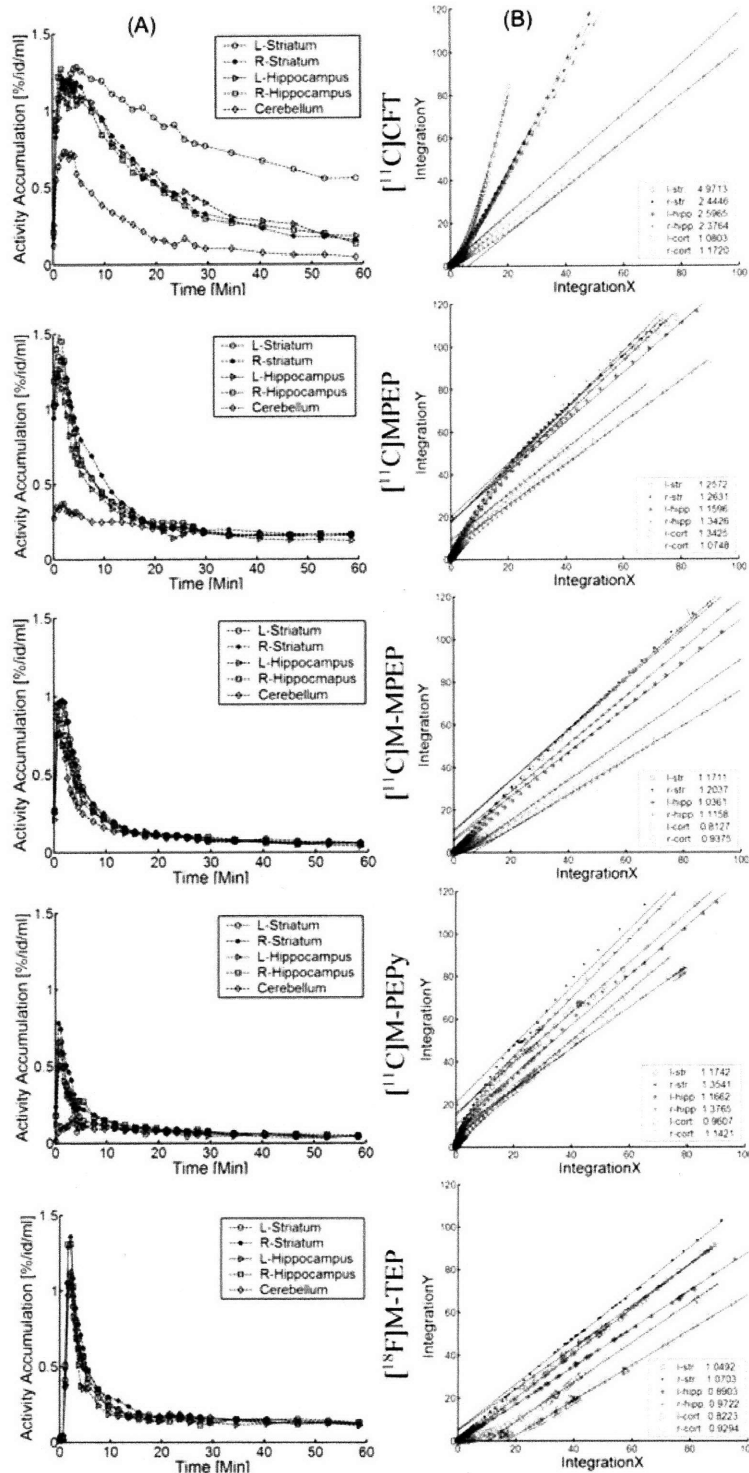
Figure 2. Graphs of Time Activity Curves (left, A) and Logan Plots (right, B).

(A) Time activity curves showing accumulation of [^{11}C]CFT, [^{11}C]MPEP, [^{11}C]M-MPEP, [^{11}C]M-PEPy, and [^{18}F]MTEP in the striatum, hippocampus, and cerebellum.

(B) Logan plots for the time activity curves in (A) show linearity: left striatum (l-str), right striatum (r-str), left hippocampus (l-hipp), right hippocampus (r-hipp), left cortex (l-cort), and right cortex (r-cort).

3.3 Binding Potential Images

Static binding potential images showing activity distribution of the dopamine transporter ligand [^{11}C]CFT and the four mGluR5 ligands [^{11}C]MPEP, [^{11}C]M-MPEP, [^{11}C]M-PEPy, and [^{18}F]MTEP are presented in Figure 3. These images were created based on the dynamic PET activity distributions shown in Figure 1. As Figure 3 shows, high binding potential values of [^{11}C]CFT can be observed in the left striatum and hippocampus, while contrastingly high binding potential values of the mGluR5 ligands can be observed in the right striatum and hippocampus. This data is consistent with the PET images in Figure 1.
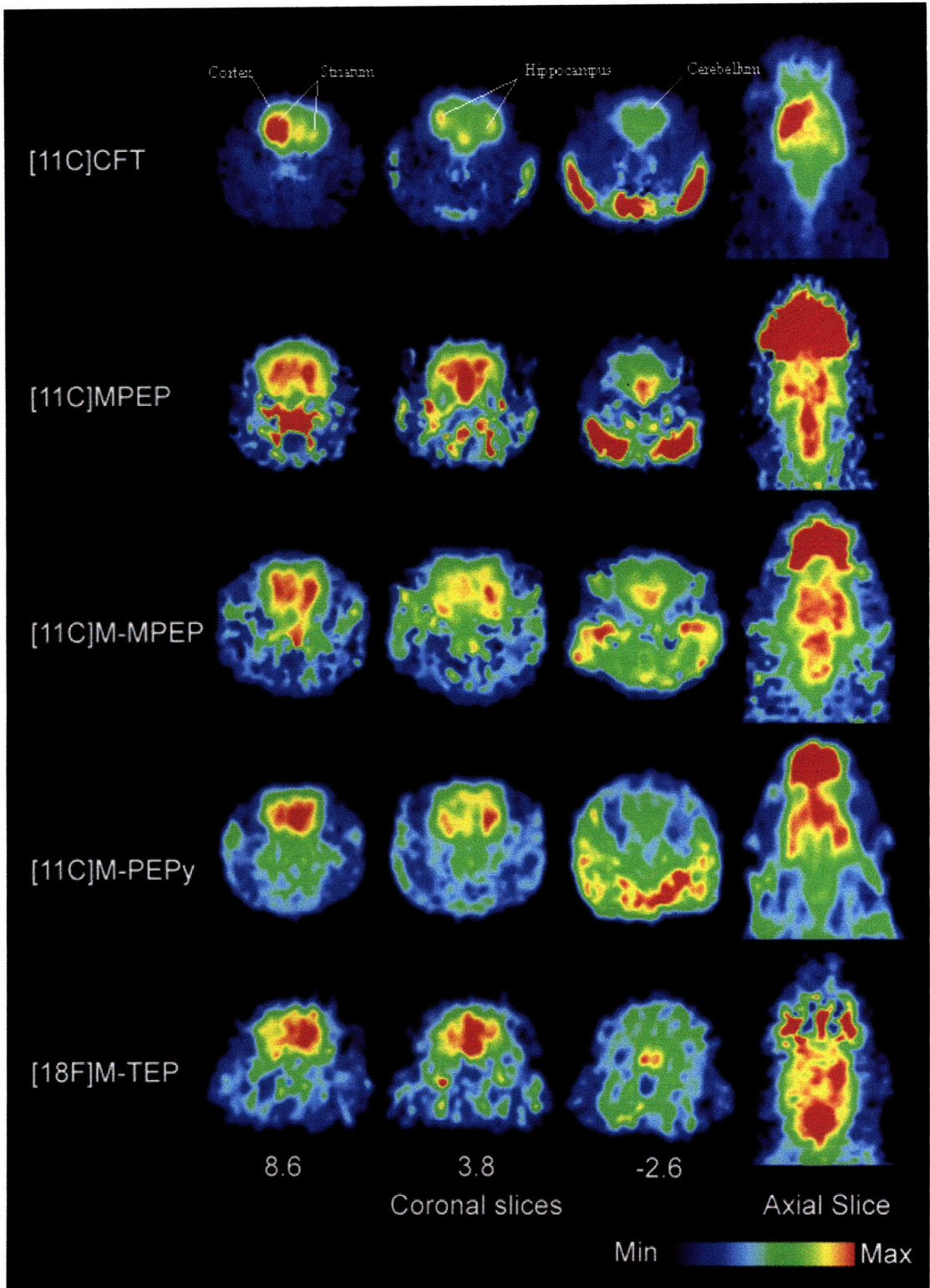
Figure 3. Binding potential images of [^{11}C]CFT, [^{11}C]MPEP, [^{11}C]M-MPEP, [^{11}C]M-PEPy, and [^{18}F]MTEP in the 6-OHDA lesioned rat brain.

The first column shows coronal images at the striatum level, the second column at the hippocampus level, the third column at the cerebellum level, and the fourth column (axial slice) at the mid-striatal level. All these levels are exactly the same levels as shown in Figure 1.

3.4 PET vs. BP Images

Comparison of Figures 1 (PET Images) and 3 (binding potential images), reproduced as Figure 4, show that the BP images of each ligand have higher contrast than the corresponding PET images of activity distribution.
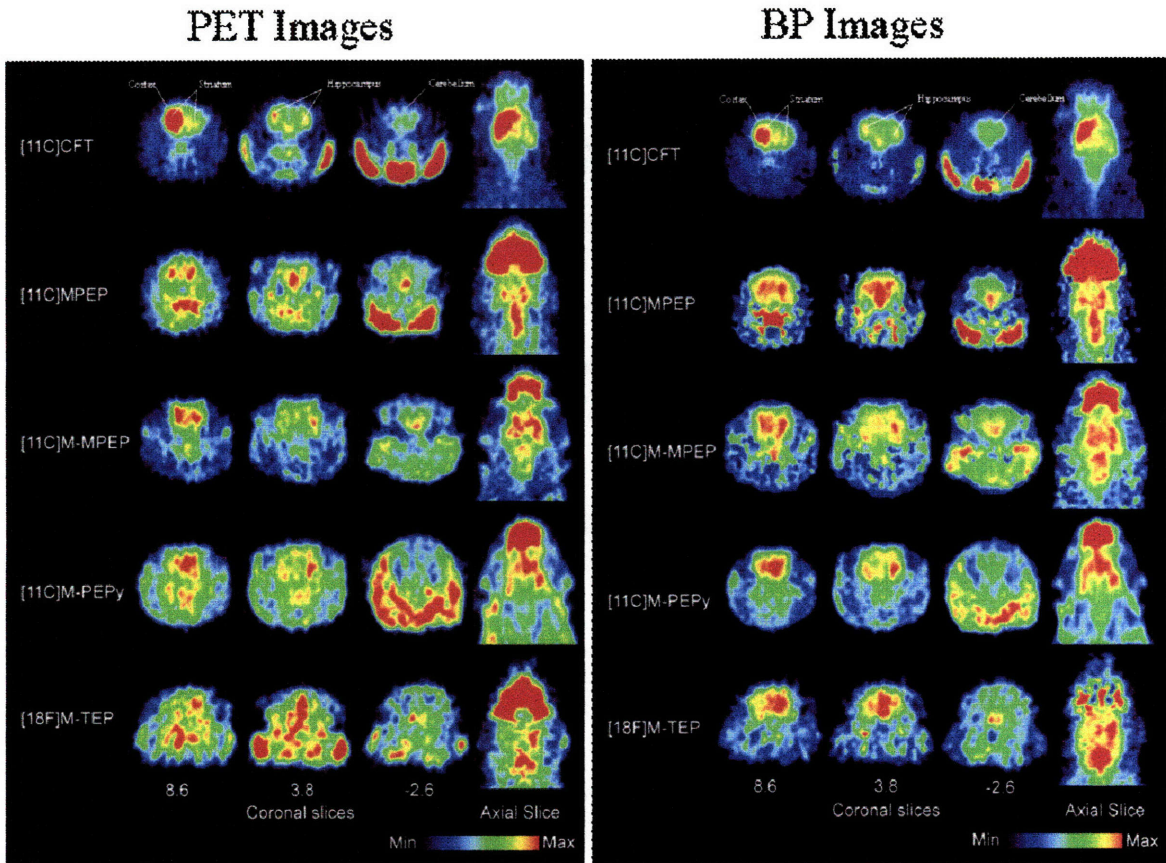
Figure 4. Comparison of PET Images (left) vs. Binding potential images (right).

3.5 Percent Differences

Percent differences were calculated for the striatum, hippocampus, and cortex (using the formula in Footnote 13) and are shown in Table 1. [^{11}C]CFT binding was decreased on the right (lesioned) striatum compared to the left side. On the same area of the lesioned striatum, three of the four tested mGluR5 ligands showed enhanced binding characteristics.

Table 1. The average percent difference of the right and left ROIs.

$[^{11}C]$CFT PET studies indicate severity of the 6-OHDA lesion. Modulation of mGluR5 function is represented as the average percent differences between the right and left striatum, hippocampus, and cortex. SD is the standard deviation.

ROIs		The average percent difference of the right and left ROIs				
		$[^{11}C]$CFT	$[^{11}C]$MPEP	$[^{11}C]$M-MPEP	$[^{11}C]$M-PEPy	$[^{18}F]$MTEP
Striatum	Mean	-35.4	4.4	-0.1	3.9	6.6
	SD	13.4	6.5	1.7	4.4	2.7
Hippocampus	Mean	-4.0	6.2	7.6	0.4	6.0
	SD	3.2	4.3	3.3	7.2	4.6
Cortex	Mean	2.6	10.2	7.4	6.9	5.8
	SD	5.9	11.3	6.2	7.4	6.4
No. PET studies for ligands		18	11	6	7	3

4. Discussion

The purpose of this study was to investigate four analogical mGluR5 PET ligands and to characterize their modulation in a 6-OHDA-induced rat model of Parkinson's disease. Characterization was accomplished by calculating the percent differences between the right (lesioned) side of the brain and the left (control) side of the brain. As shown in Table 1, the percent differences of the ligands indicate enhanced glutamatergic function in the 6-OHDA–affected regions. The percent differences of the dopamine transporter ligand indicate the severity of the 6-OHDA induced lesion in the same site. These results are consistent with the dynamic PET images of activity distribution (Figure 1) and static PET images of binding potential (Figure 3). These results concomitantly indicate a complementary role of mGluR5 receptors during or after dopaminergic degeneration. The enhanced mGluR5 function in the right side of the brain indicates mGluR5 compensation for dopaminergic loss.

Although the same dose of 6-OHDA was injected into each rat, dopaminergic degeneration was not the same in each rat (as evidenced by the large standard deviation values shown in Table 1). We do not attribute this discrepancy to the PET scanner or to the imaging techniques used. However, this result might have been caused by individual differences in of the animals, including head (brain) size and health.

Based on the percent differences of the right and left striatum, the mGluR5 ligands can be ranked in the following order, from most effective to least effective: [11C]MPEP, [11C]M-PEPy, and [11C]M-MPEP. Due to the small sample number of studies (only three animals), [18F]MTEP the ligand was not considered for ranking.

In summary, the enhanced binding of mGluR5 ligands in the 6-OHDA (right, with lesion) side of the brain indicates a complementary and compensatory role of metabotropic glutamate receptors in dopaminergic neurodegeneration. This information can be used in new designs and treatment methods of Parkinson's disease. Further research on and characterization of metabotropic glutamate receptors and their role in Parkinson's disease is still needed.

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