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ORIGINAL ARTICLE

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MPTP Animal Model of Parkinsonism: Dopamine Cell Death or Only Tyrosine Hydroxylase Impairment? – A Study Using PET Imaging, Autoradiography, and Immunohistochemistry in the Cat

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SUMMARY

Aims: 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) is a neurotoxin widely used to produce experimental models of Parkinson's disease in laboratory animals. It is believed to cause a selective destruction of substantia nigra dopamine neurons, mainly based on a large reduction of tyrosine hydroxylase (TH), the catecholamine's synthesizing enzyme. Unlike Parkinson's disease in humans, however, all animal models are able to recover more or less rapidly from the MPTP induced Parkinsonian syndrome. This raises the question as whether MPTP causes a cell death with a decrease in dopamine transporter or a simple impairment of TH. **Methods:** To respond to this question, we quantified in a cat model of Parkinson's disease (MPTP 5 mg/kg i.p. during 5 days) the dopamine transporter using positron emission tomography (PET) imaging and autoradiography of [^{11}C]PE2I and compared the data with the TH-immunoreactivity. **Results:** We found no changes in [^{11}C]PE2I PET binding either 5 or 26 days after MPTP treatment when compared to baseline levels. Similarly, there were no significant changes in [^{11}C]PE2I autoradiographic binding in the cat brain one week after MPTP treatment. In sharp contrast, MPTP treated cats exhibited severe Parkinson-like motor syndrome during the acute period with a marked decrease in TH-immunoreactivity in the striatum. **Conclusion:** These data suggest that MPTP toxicity impairs efficiently TH and that such an effect is not necessarily accompanied by significant reduction of dopamine transporter seen with *in vitro* or *in vivo* [^{11}C]PE2I binding.

Introduction

The underlying mechanisms of Parkinson's disease remain partly unknown, although several hypotheses have been proposed for linking disruption of dopaminergic pathways to oxidative stress, inflammation, or exposure to environmental agents [1–3]. In this context, the use of toxin-based animal models has given useful insights into the pathology of Parkinson's disease. The neurotoxin 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) is supposed to destroy dopamine neurons by a well-known mechanism. After systematic administration, MPTP crosses the blood–brain barrier and is converted by monoamine oxidase B to 1-methyl-4-phenyl-2,3-dihydropyridinium (MPDP), which is oxidized to 1-methyl-4-phenylpyridinium (MPP+). MPP+ is then taken up by dopamine neurons through the dopamine transporter (DAT), and acts as an inhibitor of mitochondrial complex I of the respiratory chain causing cell death. MPTP functions as a potent neurotoxin in both mice and primates, although mice appear to be less sensitive than monkeys [4–6].

The feline model constitutes an alternative model of Parkinsonism. Schneider and colleagues have shown that the cat is sensitive to the neurotoxic effects of MPTP [7–9], as MPTP administration produces a behavioral Parkinson-like syndrome, accompanied by a striatal dopamine depletion [10,11]. Unlike humans in which MPTP intoxication causes permanent Parkinson's syndrome, animal models, including the cat and monkey,

are able to recover more or less rapidly from this syndrome. This raises the question as whether MPTP causes a cell death or a simple impairment of tyrosine hydroxylase (TH) in dopaminergic neurons. To respond to this question, the use of markers other than TH to follow-up dopaminergic neurons, such as the DAT appears necessary.

Positron emission tomography (PET) enables the direct measurement of various components of the dopamine system in the animal and human brain and has served as a useful tool in monitoring neurotransmission *in vivo* and in diagnosis. However, the utility of imaging in animal models of Parkinson's disease has not been extensively evaluated, particularly in the feline model. Therefore, our aim was to evaluate the alterations in DAT during the initial phase of MPTP neurotoxicity and correlate these changes with behavioral and TH-immunohistochemical data. For this purpose, we used [^{11}C]PE2I (N-(3-iodopro-2E-enyl)-2beta-carbomethoxy-3beta-(4′-methylphenyl) nortropane), a high affinity DAT inhibitor, as tracer to evaluate the striatal DAT levels both *in vivo* using PET and *in vitro* using autoradiography in the cat.

Methods

Animal model

All experiments followed European Ethics Committee (86/6091EEC) and French National Committee (decree 87/848) directives. The experimental protocol was approved by the Ethic Committee of the University of Lyon. Every effort was made to minimize the number of animals used and any pain and discomfort.

Experiments were carried out in cats of both sexes weighing 3.2–4.1 kg, born and bred in our own animal facilities. They were implanted electrodes for behavioral EEG and sleep-wake monitoring to study the possible correlation between the MPTP-induced behavioral and sleep-wake effects and the loss of dopaminergic neurons. After baseline recordings, they were given by intraperitoneal route 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP, Sigma, St. Louis, MO, USA) at dose of 5 mg/kg i.p. for five consecutive days [12]. Cortical EEG and sleep-wake parameters were observed during 3–5 weeks after the first MPTP injection. Among these animals, six cats were used in addition to the sleep-wake study for PET imaging, autoradiographic, and immunohistochemical studies, and the data are reported in this study. EEG and sleep-wake data will be reported separately. [^{11}C]PE2I PET imaging was examined before (control), 5 or 26 days after the first MPTP injection.

Magnetic Resonance Imaging (MRI) Procedure

Before the beginning of MPTP administrations, three cats were used to build an MRI template. They were anesthetized with 2.5% isoflurane, and their head was immobilized in a stereotaxic plexiglas frame with ear bars, orbital, and hard palate pieces. MRI acquisitions (1.5-T Siemens Magnetom scanner; Siemens AG, Erglangen, Germany) consisted of a three-dimensional anatomical T_1-weighted sequence, which lasted 40 min. The anatomical volume covered the whole brain with 0.7 mm^3 voxels.

PET Procedure

The PE2I precursor was synthesized and radiolabelled by [^{11}C] CH3I O-methylation as previously described [13]. The specific activity of the injected [^{11}C]PE2I ranged from 74 to 148×10^3 MBq/μmol (2–4 Ci/μmol, at the time of injection). PET acquisitions were performed on a Siemens CTI-ECAT HR+ (Knoxville, TN, USA). The three cats were scanned twice to determine baseline values, and once after 1 week of daily injections of MPTP (5 mg/kg, i.p. $\times$5). One of the cats was also scanned after a second series of daily MPTP injections (5 mg/kg, i.p. $\times$5) and 3 weeks later.

After isoflurane anesthesia, a catheter was inserted into the forearm branch of the brachiocephalic vein. Animals were secured with a stereotaxic plexiglas frame defining the horizontal plane. A 10-min transmission scan was performed, followed by a bolus injection of 74 MBq (2 mCi) of ^{11}C-PE2I. Radioactivity was measured in series of sequential time frames of increasing duration from 30 s to 10 min for a total time of 60 min. Sinograms were normalized, attenuated, corrected for scatter and reconstructed with a filtered backprojection yielding a dynamic study of 15 volumes of $128 \times 128 \times 63$ with a voxel size of $0.86 \times 0.86 \times 2.42$ mm^3 [14].

Dynamic PET volumes were integrated and manually co-registered with MRI data (MNI-BIC Software; Montreal, QC, Canada) using a rigid body transformation with 6 degrees of freedom. A 330 mm^3 ellipse was drawn in the center of cerebellum on the MRI. The cerebellum was chosen as the region of reference as it is almost devoid of dopamine transporter binding sites [15].

Parametric images of BP (the ratio of available receptor density to receptor affinity), k_2 (the tracer's efflux in the vascular system), and R_1 (the ratio of plasma to brain transport constant) were calculated from individual voxel time–activity curves using Receptor Parametric Mapping software [16]. BP volumes were then automatically co-registered, using PET-to-PET cross-correlation with 7 degrees of freedom. The same transformation matrices were applied to parametric images of k_2 and R_1.

All PET volumes of BP were averaged into a single volume, which was then fused with the MRI to draw the regions containing BP (Figure 1), regrouped into anatomical volumes of interest (VOIs). Snyder and Niemer's stereotaxic atlas of the cat brain [17] was used as an anatomical reference to draw the right and left striatum (2×270 mm^3), and midbrain region (100 mm^3). Regional radioactivity concentration (nCi/cc) was also measured in the dynamic PET volumes for each VOI and plotted versus time.

Differences in BP between control and MPTP treated cats were assessed by non-parametric repeated measures ANOVA (Friedman's test) followed by Mann–Whitney's test for $P < 0.05$ (InStat 3.06, GraphPad Software, San Diego, CA, USA).

Autoradiography Procedure

In vitro autoradiographic studies were performed similarly for one control cat and for one MPTP pretreated cat. After euthanasia by an i.p. pentobarbital overdose, the cat was intracardiacally perfused with a Ringer solution. The brain was carefully removed and immediately frozen in 2-methylbutane cooled with dry ice at -29°C. Thirty-μm-thick coronal sections were cut

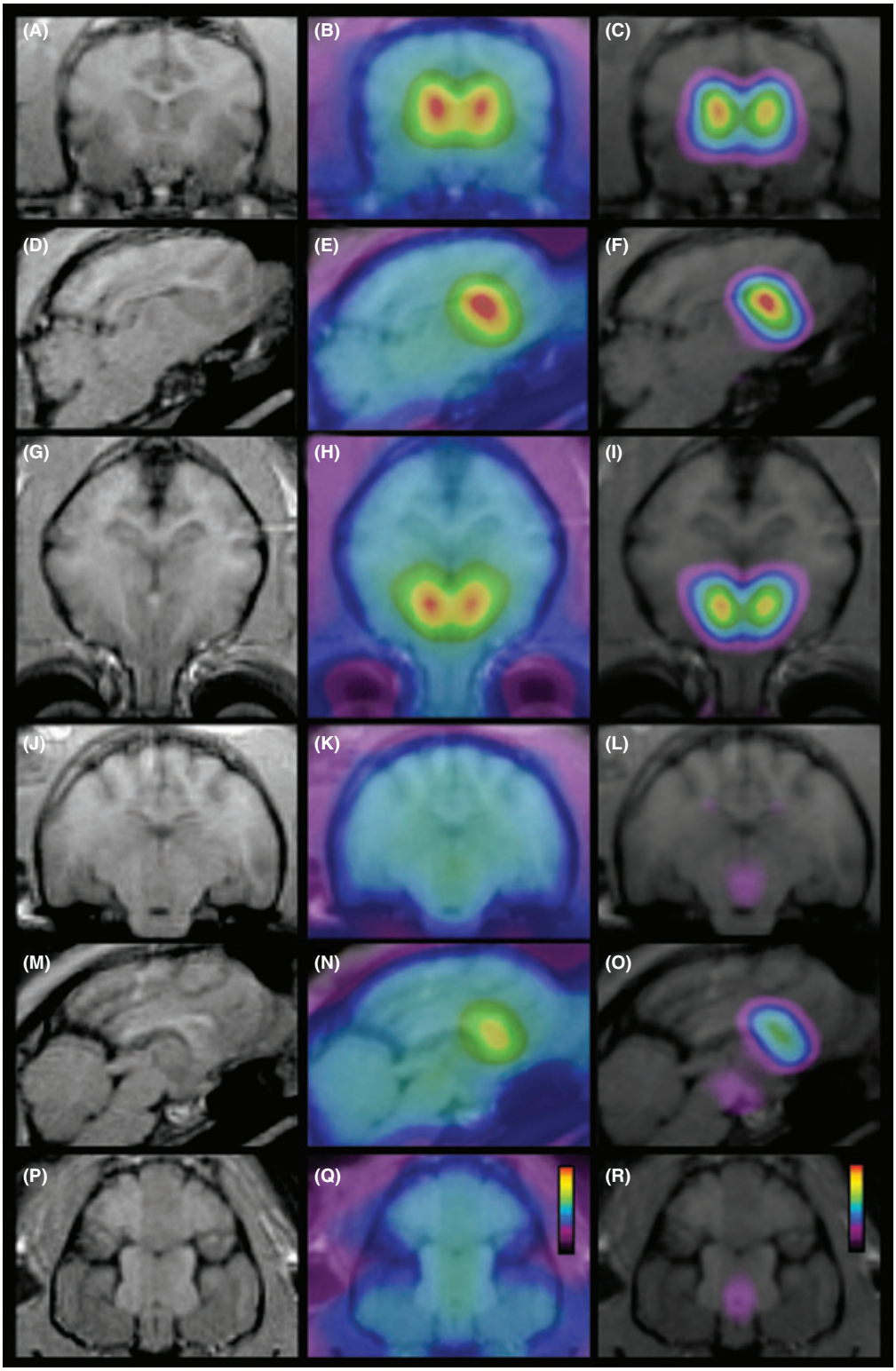

Figure 1 MRI (**A**, **D**, **G**, **J**, **M**, **P**), PET distribution of ^{11}C-PE2I binding (**B**, **E**, **H**, **K**, **N**, **Q**), and co-registered MRI/PET BP (**C**, **F**, **I**, **L**, **O**, **R**) images in the cat brain at different anatomical levels in the coronal (**A**–**C**, **J**–**L**), sagittal (**D**–**F**, **M**–**O**), and horizontal (**G**–**I**, **P**–**R**) planes. PET data were obtained from the sum of eleven scans performed in three cats (74 MBq of ^{11}C-PE2I, anesthetized with isoflurane). Pseudocolor scales range from 0 to 2000 nCi/cc and 0.5–7 in Q and R, respectively.

using a −20°C-cryostat (Microm-Microtech, Paris, France), thaw-mounted on glass slides and stored at −80°C. The day of [^{11}C]PE2I synthesis, the slides were allowed to reach ambient temperature and were then incubated 20 min in Tris phosphate-buffered saline buffer (138 mM NaCl, 2.7 mM KCl, pH 7.6) containing 37 kBq/mL (1 μCi/mL) of [^{11}C]PE2I. After incubation, the slides were dipped in cold buffer and distilled water (4°C), then dried and juxtaposed to a phosphor imaging plate for 60 min (BAS-1800 II; Fujifilm, Tokyo, Japan). Regions of interest were drawn manually using Multigauge software (Fujifilm). Measurements obtained in the caudate nucleus and putamen were normalized using the cerebellum and expressed as Standard Uptake Value.

Immunohistochemistry of Tyrosine Hydroxylase

After PET procedure, the MPTP-treated cats were processed for TH immunohistochemistry as described in a previous study [18]. Data from non-treated animals, already obtained in our laboratory, were used as control. Briefly, under deep anesthesia, cats were perfused with paraformaldheyde and brain blocks were cut using a cryostat (−22°C). Freely floating coronal sections (25 μm thick) were incubated successively with (i) a rabbit polyclonal anti-TH antibody (1:20000, for 72h at 4°C; Jacques Boy Institute, Reims, France); (ii) an anti-rabbit IgG antibody (BA-1000; Vector Laboratory, Burlingam, CA, USA); (iii) an avidin–biotin complex (Vectastain ABC Kit ref PK6100; Vector laboratory). The two latter incubations were at 4°C overnight. Finally, the immunohistochemical product was revealed using 3-3′ diaminobenzidine tetrahydrochloride (DAB; Sigma)-nickel technique.

Results

Behavior

As previously reported, MPTP-treatment (5 mg/kg. i.p. ×5 days) caused, during the acute period (up to 2 weeks), a severe Parkinson-like motor syndrome characterized by a sharp decrease in animal activity and locomotion (akinesia), and a marked hesitation to initiate movement [7,12,19]. Moreover, these cats presented a severe hypersomnia in slow wave sleep (SWS), accompanied by wake deficiency and pronounced behavioral somnolence reminiscent of excessive daytime sleepiness. During the chronic period (3rd–4thweek post-treatment), whereas the amount of waking and SWS returned to control level, paradoxical sleep showed transient increase (+30–50% of control over 2–4 days), accompanied by prolonged episode duration and narcolepsy-like episodes similar to that seen in Parkinsonian patients. Data will be presented in a separate study in preparation.

PET

After the intravenous injection of [^{11}C]PE2I, radioactivity rapidly accumulated in the brain with a high uptake of radioactivity in the striatum (Figure 1B,E,H,N) and to a lesser extent in the midbrain (Figure 1K,N,Q), which remained high during the remainder of the scan (Figure 2A–B). In contrast, after initial washout, few radioactivity remained in the cerebellum region (Figure 2A–B). This regional pattern was even more clear cut when observing the BP distribution, with high values in the striatum (Figure 1C,F, I,O), medium in the midbrain (Figure 1L,O,R), and almost nil in other brain regions.

There were no apparent changes between [^{11}C]PE2I radioactivity time curves before and after MPTP treatment (Figure 2A–B). Accordingly, the distribution and values of [^{11}C]PE2I parameters (R1, k2, and BP) were also unchanged in the striatum and midbrain of the cat following one or 2 weeks of daily injections of MPTP, or even 3 weeks later (Figure 2G).

Autoradiography

Autoradiography revealed a high density of [^{11}C]PE2I binding sites in the caudate nucleus and putamen (Figure 3A–B). In contrast, the cerebellum was almost devoid of any labeling (Figure 3C-D). There were no apparent changes in [^{11}C]PE2I binding in any region examined after 1 week of MPTP treatment (Figure 3).

Immunocytochemistry

In contrast and in consistence with previous studies [7,8,10,12], immunohistochemistry of tyrosine hydroxylase (TH) revealed a marked decrease in TH-immunoreactivity in the whole brain of the MPTP-treated animals. There was a significant decrease in the number of TH-immunoreactive cell bodies (estimated to 40–50%) and density of the labeling in the substantia nigra and adjacent midbrain dopaminergic structures either at day 6 or at days 21–26 of the MPTP treatment. The number and intensity of TH-immunoreactive varicose fibers and terminal-like dots were also sharply reduced in the striatum (see Figure 4, compare the right panel, MPTP treatment to the left one, control animal with saline injection).

Discussion and Conclusion

The cat MPTP model of Parkinsonism is interesting not only because of its known sensitivity to MPTP [7,8,10] but also because of certain similarities in the organization of the striatum between cats and humans. Furthermore, the sheer size of the animal's brain allows them to be used in studies performed with clinical PET.

MPTP treatment in cats produces major signs of motor disorders similar to those seen in Parkinson's disease. The loss of TH-positive cells and fibers in cats treated with MPTP was consistent with previous studies describing that MPTP administration during 5 days leads to a strong and long-lasting decrease in tyrosine hydroxylase (TH) immunoreactivity in the striatum [7,8,10,12]. These studies have also shown that although the ventral striatum is only modestly reinnervated by TH-positive fibers, cats spontaneously recover motor function after a few weeks [20]. Interestingly, like in the monkey, repeated exposure to MPTP in recovered cats reinstates Parkinson-like motor deficits without further decreasing the number of TH-labeled cells in most brain regions [11].

This study is the first to examine the distribution of [^{11}C]PE2I in the cat brain. Several [^{11}C]PE2I studies have demonstrated the important loss of DAT-binding sites after MPTP treatment in experimental models of Parkinsonism, like baboons, common marmoset, or cynologous monkeys [21–24].

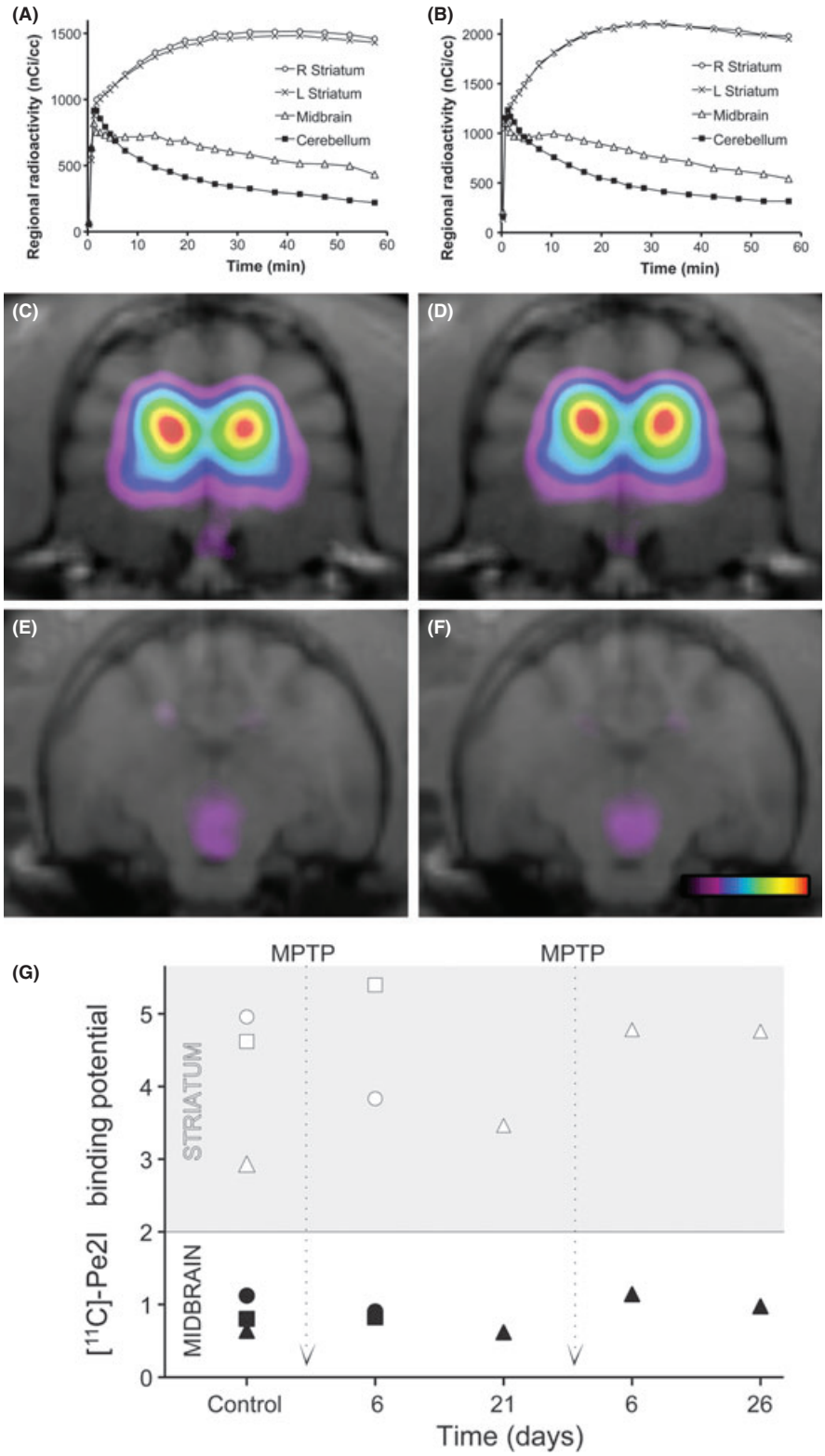

Figure 2 The time–activity curves (in **A** and **B**) are the sum of ^{11}C-PE2I binding in the striatum and cerebellum before (**A**) and after 1 week of daily injections of 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) (**B**) in the same three cats. Regional radioactivity is expressed in nCi/cc. **C**, **E** and **D**, **F** are color-coded parametric images representing ^{11}C-PE2I BP in the striatum and midbrain, as described in materials and methods, before and after 1 week of MPTP treatment, respectively. Pseudocolor scale ranges from 0.5 to 6.

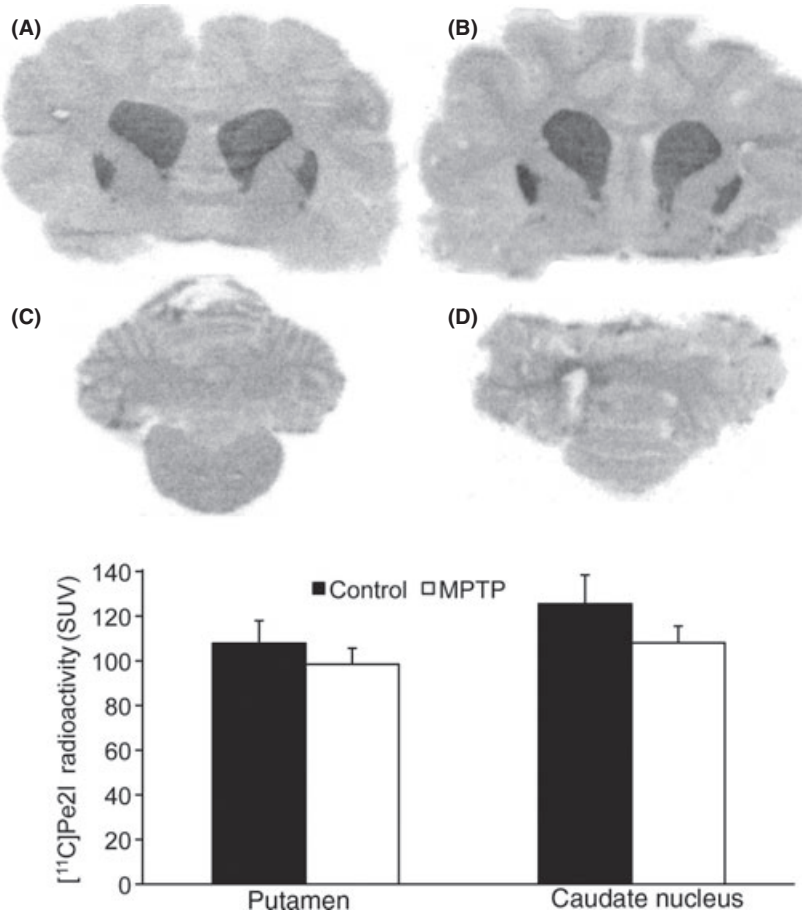

Figure 3 Autoradiographic distribution of ^{11}C-PE2I in the caudate nucleaus, putamen (**A**, **B**), and cerebellum (**C**, **D**) in a control cat (**A**, **C**) and after 1 week of daily injections of 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (**B**, **D**). Histogram representing ^{11}C-PE2I radioactivity measured in the aforementioned regions of interest.

PET imaging and autoradiography both revealed a high density of [^{11}C]PE2I binding sites, which were confined to the caudate nucleus and putamen. As the present study focuses on comparing [^{11}C]PE2I binding in cats before and after acute or chronic MPTP treatment, we took a number of methodological precautions. In this way, co-registration of PET volumes with the MRI template was crucial in ensuring the proper measurement of regions of interest. Considering the resolution of the PET camera and [^{11}C] high energy, binding potential (BP) values were probably underestimated due to partial volume effects [25]. Nevertheless, to minimize the error inherent to measuring radioactivity in a small brain region, we used a geometric template encompassing the midbrain to ensure a constant shape and size of volumes of interest between scans. Our [^{11}C]PE2I BP values should thus be considered as an index for comparison between control and treated animals rather than absolute quantitative measures.

Neither autoradiography nor PET imaging showed any significant change in [^{11}C]PE2I binding in any region examined after MPTP treatment despite large decreases in TH revealed by immunocytochemistry. Two factors could account for this result. Firstly, MPTP does not destroy a sufficient number of dopaminergic neurons in the cat brain but could transiently impair their ability to function, by down-regulating the dopaminergic function. TH-labeling could be decreased in major dopamine cells, whereas dopamine transporters could still be present [26]. This would explain the modest recovery of TH labeling observed in dopamine cells [10]. Secondly, the remaining dopaminergic neurons after MPTP treatment might compensate by rapidly upregulating dopamine biosynthesis and transporters. This hypothesis is consistent with the significant recovery in extracellular levels of DA in striatal regions [27,28] and the 60 % increase in dopamine D_2/D_3 receptor number after MPTP administration [29,30]. However, it has been also reported that dopamine transporter mRNA expression and protein levels do not increase after MPTP treatment in cat [20]. Further studies are warranted to elucidate these apparent discrepancies. Finally, as DAT-binding sites were still present after MPTP treatment, it may be concluded that MPTP toxicity efficiently impairs TH without necessarily causing cell death of dopaminergic neurons on a large scale. Thus, these data could shed some light on the relatively rapid recovery of motor function in MPTP animal models of Parkinson's disease. Further studies are warranted to determine why certain neuronal populations of dopamine neurons do not seem to succumb to the neurotoxic effects of MPTP.

In summary, the feline MPTP model of Parkinsonism is unique as it may allow the identification of relationships between dynamic processes that may be associated with compensation from a large dopamine depleting lesion. These findings may have relevance for understanding the long-term compensatory processes underlying the pre-symptomatic period of Parkinson's

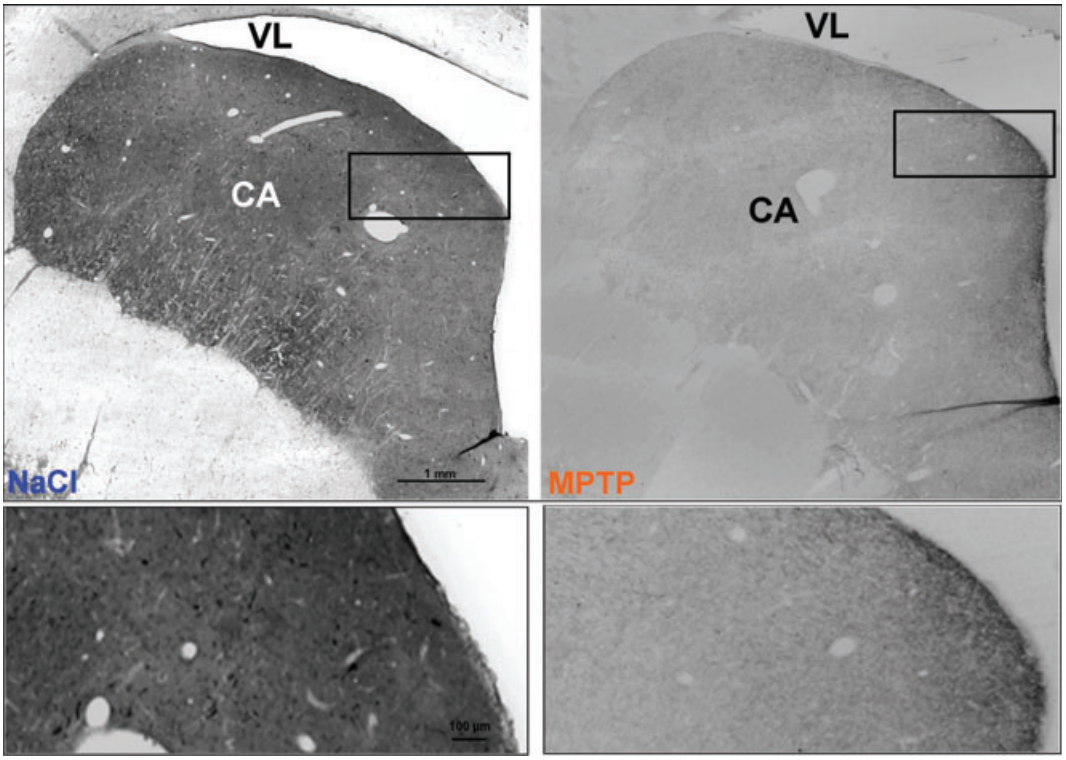

Figure 4 Photomicrographs depicting the effects of 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) (5 mg/kg, i.p. ×5 daily injection), on the ex-vivo immunoreactivity of tyrosine hydroxylase (TH) in the striatum (Caudate nucleus, CA) 3 weeks after injections. Note that the MPTP treatment strikingly decreased the density of TH-positive fibers and terminal-like dots in the CA of the MPTP-treated cats (right panel) as compared to control animals (treated with NaCl, left panel). Lower photomicrographs are higher power magnification of the boxed areas in upper photomicrographs. Other abbreviation: VL: lateral ventricle.

disease. In terms of molecular imaging, these findings highlight the fact that PET results are not systematically transferable from one animal model to another.

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Conflict of Interest

The authors declare no conflict of interest.

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