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1 **Different alterations of agonist and antagonist binding to 5-HT<sub>1A</sub> receptor**  
2 **in a rat model of Parkinson's disease and levodopa-induced dyskinesia : a**  
3 **microPET study**

4

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20 Running title: 5-HT<sub>1A</sub>R agonist and antagonist binding in Parkinson's disease and LID

21

22 Key words: 5-HT<sub>1A</sub>; Parkinson's disease; serotonin; Levodopa-induced dyskinesia; GPCR

23

24

25 **Abstract:**

26 **BACKGROUND :** The gold-standard treatment for Parkinson's disease is L-DOPA, which in  
27 the long term often leads to levodopa-induced dyskinesia. Serotonergic neurons are partially  
28 responsible for this, by converting L-DOPA into dopamine before uncontrolled release as a  
29 “false neurotransmitter”. The stimulation of 5-HT<sub>1A</sub> receptors can reduce involuntary  
30 movements but this mechanism is poorly understood.

31 **OBJECTIVES :** This study aimed to investigate the functionality of 5-HT<sub>1A</sub> receptor using  
32 positron emission tomography in hemiparkinsonian rats with or without dyskinesia induced by  
33 3-weeks daily treatment with L-DOPA. Imaging sessions were performed “off” L-DOPA.

34 **METHODS :** Each rat underwent a positron emission tomography scan with [<sup>18</sup>F]F13640, a 5-  
35 HT<sub>1A</sub>R agonist (which labels receptors in a high affinity state for agonists) or [<sup>18</sup>F]MPPF, a 5-  
36 HT<sub>1A</sub>R antagonist (which labels all the receptors).

37 **RESULTS:** There were decreases of [<sup>18</sup>F]MPPF binding in hemiparkinsonian rats in cortical  
38 areas. In dyskinetic animals, changes were slighter but also found in other regions. In  
39 hemiparkinsonian rats, [<sup>18</sup>F]F13640 uptake was decreased in the globus pallidus and thalamus.  
40 On the contralateral side, binding was increased in the insula, the hippocampus and the  
41 amygdala. In dyskinetic animals, [<sup>18</sup>F]F13640 binding was strongly increased in cortical and  
42 limbic areas, especially in the non-lesioned side.

43 **CONCLUSION :** These data suggest that agonist and antagonist 5-HT<sub>1A</sub> receptor-binding sites  
44 are differently modified in Parkinson's disease and levodopa-induced dyskinesia. In particular,  
45 these observations suggest an important involvement of the functional state of 5-HT<sub>1A</sub>R in  
46 levodopa-induced dyskinesia and emphasize the need to characterize this state using agonist  
47 radiotracers in physiological and pathological conditions.

48

## 49 **Introduction**

50

51 Parkinson's disease is a neurodegenerative disorder characterized by a wide variety of motor  
52 and non-motor symptoms. This disease affects approximately 1% of individuals older than 60  
53 years and represents a major public health problem in developed countries [1]. At the onset of  
54 the disease, the gold-standard treatment for motor symptoms remains L-3,4-  
55 dihydroxyphenylalanine (L-DOPA) [2]. This 'dopamine replacement' therapy is the most  
56 effective and the best tolerated in the short term [3-5]. However, long-term use of L-DOPA  
57 commonly leads to the development of debilitating dyskinesia known as levodopa-induced  
58 dyskinesia (LID) [6].

59 The pathological hallmark of Parkinson's disease is the loss of dopaminergic neurons in the  
60 substantia nigra pars compacta (SNc) and subsequent loss of dopamine release in the projection  
61 areas, but it is known that other monoaminergic systems are involved in the motor and non-  
62 motor symptoms. Indeed, the serotonergic system plays an important role in the  
63 pathophysiology of the disease [7-9] and the appearance of LID.

64 Although the exact mechanisms are not completely elucidated, LID is associated with the  
65 conversion of L-DOPA into dopamine by serotonergic neurons and its aberrant striatal release  
66 as a 'false neurotransmitter' by lack of autoregulatory mechanisms [10, 11]. Importantly,  
67 several studies have shown that activation of 5-HT<sub>1A</sub> receptors decreases LID [12-16], which  
68 may be attributed to the inhibition of the 5-HT system via the stimulation of 5-HT<sub>1A</sub>  
69 autoreceptors (thereby reducing the release of dopamine by 5-HT terminals in the striatum). It  
70 should be noted that a direct activation of post-synaptic 5-HT<sub>1A</sub> receptors in the striatum is also  
71 able to decrease LID [17, 18]. 5-HT<sub>1A</sub> receptor agonists have therefore been proposed as

72 promising candidates for the treatment of LID, and may also be useful to treat motor or non-  
73 motor symptoms of Parkinson's disease [19].

74 In this context, a better understanding of the pathophysiological role of this receptor in  
75 Parkinson's disease and LID seems critical to facilitate development of future treatments. To  
76 this end, several PET and autoradiography studies have been conducted using 5-HT<sub>1A</sub> receptor  
77 (5-HT<sub>1A</sub> R) radioligands [20]. However, results have been inconsistent: some studies suggested  
78 an up-regulation[21-23], whereas others suggested a loss of 5-HT<sub>1A</sub>R [24-26]. In addition, 5-  
79 HT<sub>1A</sub>R PET studies used antagonist radiotracers, such as [<sup>11</sup>C]WAY-100635 or [<sup>18</sup>F]MPPF, and  
80 not agonist radiotracers. This is important because G protein-coupled receptors (GPCRs) such  
81 as 5-HT<sub>1A</sub>R can exist in a high or a low affinity states for agonists, depending on whether they  
82 are coupled or not to their G proteins [27]. Whereas 5-HT<sub>1A</sub> receptor antagonists bind with  
83 equal affinity to coupled and uncoupled 5-HT<sub>1A</sub>R, agonists bind preferentially to the coupled  
84 receptors [28] in other words, to the active state of the receptor [29]. Thus, previous studies  
85 using antagonist radiotracers did not evaluate the functional state of 5-HT<sub>1A</sub>R in Parkinson's  
86 disease. Given that GPCR coupling is potentially altered in pathological conditions [30-32] it  
87 seems critical to investigate the active state of 5-HT<sub>1A</sub>R in order to better understand their role  
88 in Parkinson's disease and LID [33].

89 We recently developed and validated in animal the first fluorinated agonist radiotracer of 5-  
90 HT<sub>1A</sub> receptors, [<sup>18</sup>F]F13640 [34], and we have transferred it to humans as a new PET  
91 radiopharmaceutical [35]. This radiotracer is a full 5-HT<sub>1A</sub>R agonist and displays a high affinity  
92 and selectivity for 5-HT<sub>1A</sub> receptors [36, 37]

93 Here, the aim was to investigate changes of 5-HT<sub>1A</sub>R coupling in the 6-OHDA-lesioned rat  
94 model of Parkinson's disease using both a 5-HT<sub>1A</sub> antagonist radiotracer, [<sup>18</sup>F]MPPF, and the

95 agonist radiotracer [<sup>18</sup>F]F13640. We also studied the functional state of 5-HT<sub>1A</sub>R in LID, using  
96 HPK rats that were chronically treated with L-DOPA.

97

## 98 **Methods**

99

### 100 *Animals*

101 Twenty-eight male Sprague-Dawley adult rats (Charles River laboratories, France) of  
102  $364.92 \pm 60.71$  g were used. Animals were housed in standard temperature and humidity  
103 conditions with a 12h/12h light/dark cycle. Food and water were provided *ad libitum*. All  
104 experiments were performed in accordance with the European guidelines for care of laboratory  
105 animals (2010/63/EU) and approved by the University of Lyon review board.

106

### 107 *Unilateral 6-OHDA Lesions in the Medial Forebrain Bundle*

108 Rats were anesthetized using isoflurane. Prior to surgery, buprenorphine (0.05 mg/kg, s.c.) was  
109 administered for peri- and post-surgical analgesia. Lidocain was used for local analgesia.

110 Rats were lesioned in the right medial forebrain bundle (MFB) according to the Paxinos and  
111 Watson's atlas [38] at coordinates AP=-4.0 mm, L=-1.3 mm, DV=-7.6 mm relative to bregma  
112 and the dural surface by infusing 6-OHDA (Sigma Aldrich, MO, USA) (14 µg/4 µL) dissolved  
113 in saline containing ascorbic acid (0.02%). A volume of 4 µL was injected over 5 min, leaving  
114 the microsyringe in situ for further 5 min.

115 After 3 weeks, the rats were challenged with apomorphine (0.05 mg/kg, s.c.; Sigma-Aldrich)  
116 and turning behavior assessed over 30min. The rats making >10 net full turns contralateral to

117 the lesion were used in further experiments. This value was chosen in order to ensure a striatal  
118 dopamine depletion of at least 90% with no risk of false positives, as virtually no rotations are  
119 observed following apomorphine administration in the absence of such a lesion [39].

120

### 121 *Development of L-DOPA-induced motor behaviors*

122 For the study of LID, 6-OHDA lesioned rats received chronic daily treatment with L-DOPA (6  
123 mg/kg) and benserazide (12 mg/kg) for 3 weeks in order to induce stable reproducible abnormal  
124 involuntary movements. After this induction phase, the rats were maintained with 2 injections  
125 of L-DOPA per week for the duration of the experiments (L-DOPA was not administered the  
126 days of imaging sessions, so that there was at least a 24 hour interval between an injection and  
127 the PET scan). Induction of LID was assessed by observing for one minute each rat at t: 20, 40,  
128 60, 80, 100 120, 140, 160 and 180 min post L-DOPA administration, at day 1 (D1), day 7 (D7),  
129 day 14 (D14) and day 22 (D22) of treatment, using the following scale:

130 0: Absence of dyskinesia (normal)

131 1: Orolingual dyskinesia

132 2: Orolingual + moderate limb and axial dyskinesia

133 3: Orolingual + pronounced limb and axial dyskinesia.

134

### 135 *PET/CT imaging*

136 Animals were divided in three groups: 6-OHDA-lesioned rats (HPK; n=7), 6-OHDA-lesioned  
137 rats with LID (HPK-LID; n=9) and control rats that had not been lesioned (CTRL ; n=12).  
138 Animals either underwent 2 different PET scans with [<sup>18</sup>F]MPPF and [<sup>18</sup>F]F13640 (with a  
139 interval ranging from 2 to 19 days, and average of 7.7 days), or a single scan with either  
140 [<sup>18</sup>F]MPPF or [<sup>18</sup>F]F13640. Animals were scanned “off L-DOPA”. For each scan, rats were

141 anesthetized with 4–5% isoflurane for 5 min (induction phase), and a catheter was placed into  
142 their caudal vein. Anesthesia was then lowered to 2% isoflurane during the acquisition on the  
143 PET/CT (Inveon<sup>®</sup>, Siemens, Knoxville, TN, USA), with monitoring of the respiratory rate using  
144 a pressure sensor. The acquisition started with a CT image acquisition for 10 min, followed by  
145 the intravenous injection of [<sup>18</sup>F]MPPF ( $14.01 \pm 2.30$  MBq) or [<sup>18</sup>F]F13640 ( $12.14 \pm 2.68$   
146 MBq). The total duration of the scan was 60 min. The images were reconstructed in three  
147 dimensions in a series of 24 sequential frames of increasing duration from 10 s to 5 min.  
148 Regions of Interest (ROIs) were automatically delineated using a multi-atlas dataset [40], after  
149 manual positioning of the PET images on an anatomical MRI template using the software  
150 Inveon Research Workplace (IRW, Siemens). Distribution volume ratio (DVR) was estimated  
151 using Logan graphical method with reference region (cerebellum) calculated from 20 to 60 min  
152 in each region of interest (ROI) [41].

153 Concerning the voxel-based analysis, data processing was carried out using the statistical  
154 parametric mapping software (SPM12, Wellcome Trust Center for Neuroimaging, London;  
155 UK). Parametric images of Binding Potential Non-displaceable ( $BP_{ND}$ ) were generated for all  
156 rats using the Logan graphical method with reference region (cerebellum) calculated from 20  
157 to 60 min. Individual PET images were realigned and spatially normalized on a custom PET  
158 template for each radiotracer which was coregistered on an anatomical MRI template. The  
159 quality of the registration was visually checked between all images to verify that they fitted  
160 well together and matched the MRI template in terms of cortical boundaries. Each PET volume  
161 was smoothed using an isotropic Gaussian filter [1.4 x 1.4 x 1.4 mm].

162

163 *Statistical analyses*



164 For the comparisons of DVR in the regions of interest, a two-way ANOVA with Tukey's  
165 multiple comparisons test was performed ( $p < 0.05$ , two-tailed).

166 The comparisons of parametric images between the different groups of animals were performed  
167 voxel by voxel using a t test analysis with a significance threshold set at  $p < 0.01$  (uncorrected).

168

## 169 **Results**

170

### 171 *Development of L-DOPA-induced motor behaviors*

172 Six rats chronically treated with L-DOPA were scored as dyskinetic during the 60 min post L-  
173 DOPA administration (peak effect):  $2.3 \pm 0.2$ ,  $2.7 \pm 0.2$ ,  $2.5 \pm 0.2$  and  $2.8 \pm 0.2$  (average  
174 dyskinetic score  $\pm$  SEM, at D1, D7, D14 and D22, respectively). The other rats from the group  
175 were also confirmed as dyskinetic but not systemically quantified.

176

### 177 *PET imaging of total 5-HT<sub>1A</sub> receptors using [<sup>18</sup>F]MPPF*

178 [<sup>18</sup>F]MPPF binding was compared between hemiparkinsonian rats with or without dyskinesia  
179 and control rats (Fig. 1). At the voxel level, [<sup>18</sup>F]MPPF BP<sub>ND</sub> was significantly lower in  
180 hemiparkinsonian rats versus controls in various cortical areas, with few differences between  
181 the hemispheres (Fig. 1A). On the contrary, a local significant increase of [<sup>18</sup>F]MPPF BP<sub>ND</sub> was  
182 found in the contralateral orbital cortex. In the ROIs (Fig. 1D), the most significant decreases  
183 of distribution volumes were found in the right and left hippocampus (-11%,  $p < 0.0001$  and -  
184 8.5%,  $p = 0.0009$  respectively). In the limbic system, the ipsilateral side of the amygdala was  
185 also affected (-8.7%,  $p = 0.0407$ ). Other ROIs with a decreased [<sup>18</sup>F]MPPF binding were cortical

186 and bilateral (ranging from -15 to -11% in the ipsilateral and from -9 to -11% in the contralateral  
187 side), including the cingulate cortex, frontal cortex, occipital cortex and parietal cortex, but not  
188 the temporal cortex, where only the contralateral side was affected.

189 In the dyskinetic HPK rats, [<sup>18</sup>F]MPPF uptake was also modified in several areas (Fig. 1B).  
190 Fewer changes were found in the cortex, and were mainly located in the right somatosensory  
191 and motor cortices. Other decreases were found mainly in the limbic system: medial septum,  
192 lateral entorhinal cortex, and ventral hippocampus. [<sup>18</sup>F]MPPF uptake was also decreased in  
193 the right thalamus and the brainstem. Significant increases were found in the orbital cortex,  
194 similarly to HPK rats, but bilaterally. In the ROIs, significant decrease of DVR were found in  
195 ipsilateral side only in the hippocampus, the parietal cortex and the occipital cortex and ranged  
196 from -5 to -9%.

197 We also compared [<sup>18</sup>F]MPPF binding between HPK and HPK-LID rats (Fig. 1C). [<sup>18</sup>F]MPPF  
198 BP<sub>ND</sub> was significantly higher in non-dyskinetic animals in some areas of the limbic system  
199 (the medial septum and the entorhinal cortex), the ~~median~~-raphe (median and magnus) and the  
200 dorsal thalamus (in green). On the contrary, [<sup>18</sup>F]MPPF BP<sub>ND</sub> was higher in dyskinetic animals  
201 in parts of the parietal and occipital cortex, and the medial deepest part of the cerebellum (in  
202 brown). Results from the ROIs analysis also showed that [<sup>18</sup>F]MPPF uptake was higher in both  
203 sides of the hippocampus in the dyskinetic animals (Fig. 1D).

204

#### 205 *PET imaging of 5-HT<sub>1A</sub> receptors in high-affinity state using [<sup>18</sup>F]F13640*

206 Binding potentials of [<sup>18</sup>F]F13640, reflecting changes in 5-HT<sub>1A</sub> receptors in the high-affinity  
207 state for agonists, were compared between hemiparkinsonian rats with or without dyskinesia  
208 and control rats (Fig. 2).

209 When comparing HPK and control rats (Fig. 2A), the voxel-based analysis revealed a  
210 significant decrease of [<sup>18</sup>F]F13640 binding potential in a large cluster of the ipsilateral  
211 hemisphere, spreading from the globus pallidus to the thalamus. A similar but smaller cluster  
212 was found on the contralateral side. HPK rats also displayed a higher [<sup>18</sup>F]F13640 binding in  
213 several limbic areas of the contralateral hemisphere, spreading from the insular cortex to the  
214 piriform cortex, the entorhinal cortex and parts of the hippocampus. When comparing the  
215 distribution volume ratios in the different regions of interest (Fig. 2D), we only found a  
216 significant decrease in the right thalamus in HPK rats versus control rats (-11.5%, p=0.0157).

217 Significant decreases of [<sup>18</sup>F]F13640 binding in HPK-LID rats were fewer than HPK rats (Fig.  
218 2B); small clusters were found rostrally in the ventral thalamus, and caudally in the left  
219 substantia nigra, and parts of the cerebellum. On the contrary, many areas displayed a  
220 significant increase of binding potential. Similarly to HPK rats, most significant increases were  
221 located in areas of the limbic system, such as the piriform and entorhinal cortices, the amygdala  
222 and the ventral hippocampus. Other cortical regions were also affected, such as the prefrontal  
223 cortex and widespread areas of the temporal and parietal cortices. Other small increases were  
224 found in the hypothalamus and the brainstem. In general, increases were observed in both sides  
225 of the brain but were more significant in the contralateral hemisphere. Such increases were also  
226 significant when comparing the DVR between dyskinetic and control animals in several regions  
227 of interest (Fig. 2D), namely the left hippocampus (+9.9%, p=0.0249), the right and left  
228 amygdala, cingulate cortex, and temporal cortex (ranging from +11 to +12% in the ipsilateral  
229 side and from +12 to +14% in the contralateral side).

230 We also compared voxel by voxel [<sup>18</sup>F]F13640 binding between HPK and HPK-LID rats (Fig.  
231 2C). [<sup>18</sup>F]F13640 binding was significantly higher in dyskinetic animals (in brown) in various  
232 cortical areas in both hemispheres and the right striatum and thalamus. On the contrary,  
233 [<sup>18</sup>F]F13640 binding was higher in non-dyskinetic animals (in green) in the left substantia nigra

234 and the paraflocculus of the cerebellum. In the regions of interest (Fig. 2D), DVR were higher  
235 in dyskinetic compared to non-dyskinetic rats in the right striatum (+12.2%, p=0.0281), the  
236 right cingulate cortex (+10.6%, p=0.0321), and the right frontal cortex (+12.6%, p=0.0195).

237

## 238 **Discussion**

239

### 240 *5-HT<sub>1A</sub>R as a promising therapeutic target for Parkinson's disease?*

241 In addition to the dopaminergic system, it has been compellingly demonstrated that the  
242 serotonergic system is involved in the pathophysiology of Parkinson's disease, whether in terms  
243 of motor symptoms [24]; [42] or non-motor symptoms such as cognitive impairment [43] and  
244 mood disorders [44], but also in the etiology of LID [45, 46]. In this context, the 5-HT<sub>1A</sub>R  
245 appears to be a promising therapeutic target: in addition to having a potential therapeutic effect  
246 in treating motor [47-50] and cognitive [51] symptoms or mood disorders [52-55] associated  
247 with Parkinson's disease, 5-HT<sub>1A</sub> agonists are highly effective for treating LID in preclinical  
248 models [13, 14, 16, 18, 56-61]. The main proposed mechanism for their antidyskinetic effects  
249 is the stimulation of autoreceptors in the raphe nuclei. However, striatal 5-HT<sub>1A</sub>R may also play  
250 a role in control of LID [17, 62]. There are also discrepancies regarding the effect of 5-HT<sub>1A</sub>  
251 agonists on motor symptoms of Parkinson's disease [63-65]. Few clinical studies have been  
252 conducted with 5-HT<sub>1A</sub> agonists to treat LID, and reported either promising results [66-68] or  
253 worsening of parkinsonian features in a high proportion of patients [69].

254 Therefore, more information is needed to understand the mechanism of action of 5-HT<sub>1A</sub>  
255 agonists in treating LID and how to diminish dyskinesia without worsening motor symptoms  
256 in patients. Indeed, little is currently known about the impact of Parkinson's disease and LID  
257 on 5-HT<sub>1A</sub>R levels and function.

258

259 *The contribution of PET imaging in deciphering the involvement of 5-HT<sub>1A</sub>R in Parkinson's*  
260 *disease*

261 We recently reported the radiosynthesis, preclinical validation [34] and first-in-man  
262 administration [35] of the first highly selective 5-HT<sub>1A</sub>R full agonist PET radiotracer  
263 [<sup>18</sup>F]F13640. Unlike antagonist radiotracers of GPCRs, agonists radiotracers are known to bind  
264 preferentially to the receptors in a high-affinity state for agonists, that is, to receptors that are  
265 fully functional and accessible to the endogenous neurotransmitter [70-73]. We therefore  
266 investigated changes in 5-HT<sub>1A</sub>R in a rat model of Parkinson's disease using [<sup>18</sup>F]F13640 in  
267 comparison with an antagonist radiotracer, [<sup>18</sup>F]MPPF. Furthermore, changes of 5-HT<sub>1A</sub>R  
268 levels and functionality were also studied in rats that were chronically treated with L-DOPA to  
269 elicit LID. Importantly, unlabeled F13640 (also known as befiradol or NLX-112) has shown  
270 promising antidyskinetic properties in rat, marmoset and macaque models of Parkinson's  
271 disease [14, 57, 59]. Therefore, the binding of [<sup>18</sup>F]F13640 points directly to the population of  
272 5-HT<sub>1A</sub>R that are targeted by this antidyskinetic 5-HT<sub>1A</sub> receptor agonist and is likely to provide  
273 important information regarding the therapeutic mechanism of this class of drugs.

274 Previous studies using antagonist radiotracers have shown disparate results. In rats that were  
275 unilaterally injected with 6-OH-DA in the striatum, binding potential of [<sup>18</sup>F]Mefway was  
276 significantly lowered in the hippocampus [74, 75]. In cynomolgus monkeys treated with 1-  
277 methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP), [<sup>18</sup>F]MPPF binding was increased in  
278 various structures at early stages, whereas fewer changes were found in the fully symptomatic  
279 state [26]. An autoradiography study using [<sup>3</sup>H]WAY-100635 showed decreases of 5-HT<sub>1A</sub>R  
280 binding in the external layers and increases in the middle layers of the premotor and motor  
281 cortices of MPTP-lesioned macaques [21, 22]. 5-HT<sub>1A</sub>R levels were also investigated in human  
282 studies using 5-HT<sub>1A</sub> antagonist radiotracers. In 2003, Doder et al. reported a decrease of  
283 [<sup>11</sup>C]WAY-100635 binding potential in the raphe of parkinsonian patients, which was

284 significantly correlated with the tremor score [24]. Another study using [<sup>18</sup>F]MPPF showed  
285 decreases of 5-HT<sub>1A</sub>R binding in patients, depending on the comorbidity with depression [26].  
286 A postmortem autoradiography study reported an increased binding of [<sup>3</sup>H]WAY-100635 in  
287 the temporal cortex of patients [21]. These data show a complex involvement of 5-HT<sub>1A</sub>R in  
288 the pathophysiology of Parkinson's disease. The variability of these findings may be partly due  
289 to different species and models, but also by factors such disease progression, the development  
290 of dyskinesia or the chronic effects of L-DOPA treatment by itself. Indeed, long-term treatment  
291 with L-DOPA produces marked changes in the 5-HT system [76], as shown by a previous study  
292 showing increased tritiated WAY-100635 binding in MPTP-lesioned macaques chronically  
293 treated with L-DOPA compared with untreated macaques [22].

294 Once again, it should be emphasized that all the above studies used antagonist radiotracers to  
295 label 5-HT<sub>1A</sub>R: such tracers cannot characterize underlying changes in receptor-G-protein  
296 coupling state. In contrast, the use of the innovative agonist radiotracer, [<sup>18</sup>F]F13640, allowed  
297 us for the first time to examine changes in active state 5-HT<sub>1A</sub>R in the present model of  
298 Parkinson's disease.

299  
300 *PET imaging of high-affinity state 5-HT<sub>1A</sub> receptors allows revisiting the pathophysiology of*  
301 *LID*

302 The main finding of this study is ~~We found~~ that the changes observed of the population of 5-  
303 HT<sub>1A</sub>R in the active (i.e. G-protein-coupled) state, as determined by [<sup>18</sup>F]F13640 binding, were  
304 completely distinct from the changes in the whole population of receptors, as visualized using  
305 [<sup>18</sup>F]MPPF.

306 Using [<sup>18</sup>F]MPPF, 5-HT<sub>1A</sub>R binding appeared to be decreased in cortical areas only in HPK  
307 animals. In dyskinetic animals, the lower cortical binding was more restricted to the  
308 somatosensory, motor and entorhinal areas, but [<sup>18</sup>F]MPPF uptake was also lowered in

309 subcortical areas such as the thalamus and the raphe. For both pathological groups, few  
310 increases were found and restricted to the orbital cortex.

311 Such changes occurring with a 5-HT<sub>1A</sub>R antagonist radiotracer are unlikely to be related to the  
312 functional state of receptors as they are known to bind equally to the coupled or uncoupled  
313 receptors [28], or to the competition with endogenous serotonin as [<sup>18</sup>F]MPPF is poorly  
314 sensitive to supraphysiological changes in serotonin concentration [34]. Therefore, cortical  
315 decreases of [<sup>18</sup>F]MPPF binding potential could be related to an altered affinity for receptors or  
316 to a lower density of 5-HT<sub>1A</sub> receptors, which could be related to various non-motors symptoms  
317 of Parkinson's disease such as cognitive impairment, depression and anxiety [43, 44], as well  
318 as to motor and sensory deficits, since the somatosensory and motor cortices also displayed a  
319 decreased uptake. Another worthy observation is that in the raphe, [<sup>18</sup>F]MPPF binding was  
320 decreased in dyskinetic rats, which would suggest a decreased serotonergic autoinhibition that  
321 could be related to the dyskinetic behavior (as conversely, the stimulation of 5-HT<sub>1A</sub>  
322 autoreceptors is able to block LID). Interestingly, the local increase in the ventral orbital cortex  
323 is consistent with a previous autoradiography study using a 5-HT<sub>1A</sub> antagonist radiotracer that  
324 showed an increased binding in the most internal parts of the frontal cortex in parkinsonian  
325 primates that were also chronically treated with L-DOPA [21], which was suggested to be  
326 linked to the visual hallucinations symptoms.

327 Numerous changes of [<sup>18</sup>F]F13640 uptake were also found in HPK and dyskinetic rats. These  
328 changes are more difficult to interpret but also more reflective of the pathophysiological  
329 involvement of 5-HT<sub>1A</sub>R, as contrary to antagonists, agonists bind preferentially to the active  
330 state, i.e., G-protein coupled state of receptors, [28, 77]. This means that an increased proportion  
331 of receptors in the uncoupled state could lead to a decrease in [<sup>18</sup>F]F13640 binding. Another  
332 important consideration is that [<sup>18</sup>F]F13640 was shown to be very sensitive to the competition  
333 with endogenous serotonin [34], therefore a local increase in serotonin concentration could also

334 lead to a decrease in [<sup>18</sup>F]F13640 binding. As seen in the voxel analysis, [<sup>18</sup>F]F13640 binding  
335 modifications were spatially distinct from that of [<sup>18</sup>F]MPPF, therefore we propose that they  
336 were mainly due one or both of these mechanisms (intracellular coupling or extracellular  
337 serotonin concentration) rather than changes in the density of receptors (which should also be  
338 observed using [<sup>18</sup>F]MPPF).

339 In HPK rats, [<sup>18</sup>F]F13640 uptake was decreased in the globus pallidus and the thalamus,  
340 especially in the lesioned side. There might be an increased serotonergic innervation in these  
341 areas, which would be consistent with a recent study in MPTP-lesioned cynomolgus monkeys  
342 showing a 2-fold increase of serotonergic axon varicosities in the globus pallidus [78].  
343 Alternatively, 5-HT<sub>1A</sub>R coupling state might be altered in this region, which is supported by  
344 very recent findings in 6-OHDA lesioned rats showing that 5-HT<sub>1A</sub> agonists effects are  
345 decreased in the entopeduncular nucleus (which is analogous to the internal part of the globus  
346 pallidus) compared to control rats [79]. In the contralateral side, [<sup>18</sup>F]F13640 uptake was  
347 increased in limbic areas or limbic-related areas such as the insula, the entorhinal cortex, and  
348 the amygdala and the ventral hippocampus. Such increases could be due to the loss of  
349 serotonergic neurons in Parkinson's disease [80] as they were observed in areas richly  
350 innervated by 5-HT, although it is unclear why they were more pronounced in the non-lesioned  
351 side. This deserves more investigation in the future to understand if these increases are the  
352 signature of a compensatory mechanism and how they are involved in non-motor symptoms of  
353 Parkinson's disease; given that they occur in limbic regions, they may be linked to the cognitive  
354 and mood symptoms.

355 Interestingly, dyskinetic animals displayed more pronounced increases of [<sup>18</sup>F]F13640 binding  
356 in the same regions as non-dyskinetic HPK animals but that also spread to cortical areas, and  
357 to the ipsilateral side of the brain. These observations are consistent with the known  
358 involvement of the serotonergic system in LID and could be due to a possible decrease of 5-HT



359 basal levels in many regions in dyskinetic animals. The decreased 5-HT concentration would  
360 acutely result in a decreased competition towards 5-HT<sub>1A</sub>R and higher binding of [<sup>18</sup>F]F13640,  
361 but it can also be expected that a chronic depletion of 5-HT release would result in an  
362 overexpression of 5-HT<sub>1A</sub>R in the high-affinity state as a compensatory mechanism due to the  
363 chronic lack of stimulation by 5-HT. Indeed, it has been shown in 6-OH-DA-lesioned rats that  
364 L-DOPA decreases extracellular concentration of serotonin in the prefrontal cortex and  
365 hippocampus after acute administration [81] and decreases basal extracellular 5-HT levels in  
366 the PFC, striatum, hippocampus and substantia nigra after chronic administration [82]. Deficits  
367 in 5-HT release can arise from several mechanisms [83]: first, after decarboxylation of L-DOPA  
368 in 5-HT neurons, dopamine competes with 5-HT for storage into vesicles and exocytotic release  
369 [10, 84]; secondly, L-DOPA treatment can induce oxidative stress that is known to induce 5-  
370 HT neuron loss *in vivo* in HPK rats [85]. Our data are consistent with the reported deleterious  
371 effect of chronic L-DOPA treatment on 5-HT system, that would have repercussions at the post-  
372 synaptic level: the regions where increased levels of active 5-HT<sub>1A</sub>R were observed are known  
373 to be highly innervated by 5-HT neurons, probably reflecting a compensatory phenomenon  
374 following the lack of stimulation of 5-HT<sub>1A</sub>R by extracellular serotonin. Questions remain to  
375 be elucidated regarding some regions where [<sup>18</sup>F]F13640 binding was lower, such as the left  
376 substantia nigra. Although one study reported that chronic treatment with L-DOPA also  
377 decreased 5-HT levels in this region [82], another study reported increased 5-HT release in  
378 substantia nigra in awake rats chronically treated with L-DOPA [86]. Thus, L-DOPA  
379 administration may have distinctive effects on 5-HT neurotransmission in this specific region,  
380 as suggested by a microdialysis study which showed a strong increase of 5-HT release (13-fold)  
381 after local injection of L-DOPA in substantia nigra [87]. Anyway, only the contralateral side of  
382 substantia nigra was affected in our study, which suggests that dopamine neurons must be  
383 involved in this local decrease. Taken together, these findings show that the high-affinity state

384 of 5-HT<sub>1A</sub>R is even further modified by chronic L-DOPA treatment and dyskinesia, which may  
385 be related to the non-motors symptoms that can be related to the serotonergic projections areas  
386 such as the limbic system and prefrontal cortex (that is, mainly cognitive processes, anxiety and  
387 mood disorders). Indeed, there is evidence that L-DOPA can worsen these symptoms in the  
388 long term [88].

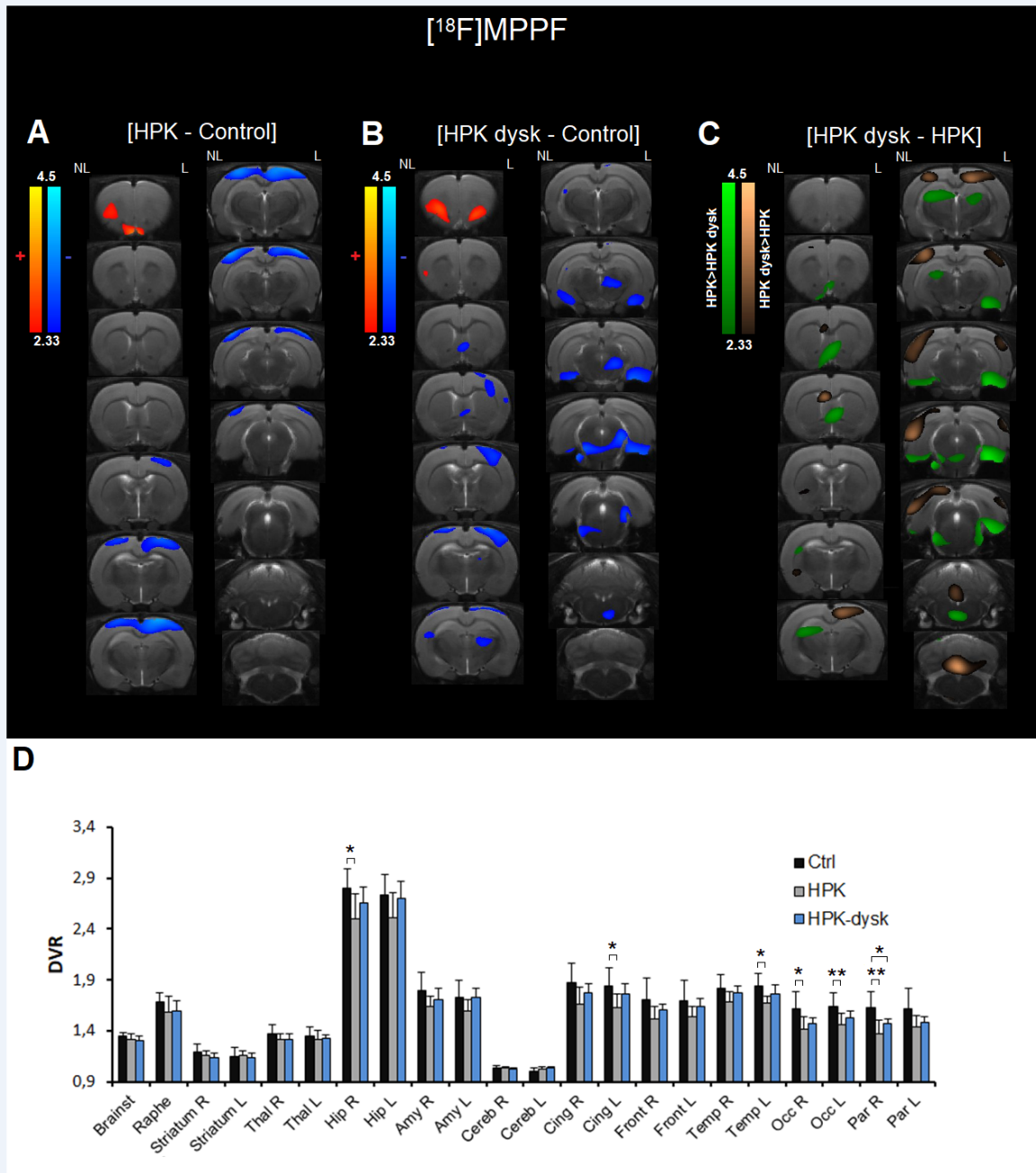
389 Another important result of our study is the increase of [<sup>18</sup>F]F13640 uptake in the lesioned  
390 striatum in dyskinetic animals compared to untreated animals. This could be explained by a  
391 lower serotonergic innervation of the lesioned striatum in dyskinetic animals, resulting in higher  
392 binding of the radiotracer, but this hypothesis is contradicted by a previous study which showed  
393 that L-DOPA chronic treatment in HPK rats results in a higher SERT binding density in the  
394 lesioned striatum [89]. Therefore, this is more likely reflecting a switching of a striatal  
395 population of 5-HT<sub>1A</sub>R in the high-affinity state, suggesting a hypersensitivity of 5-HT<sub>1A</sub>R in  
396 the lesioned side of the striatum.

397 As previously discussed, changes occurring at the level of presynaptic serotonergic  
398 transmission could explain some of our results with [<sup>18</sup>F]F13640. In the future, it would be  
399 valuable to evaluate the changes of [<sup>18</sup>F]F13640 along with the extracellular 5-HT concentration  
400 and the level of serotonin transporter. This is also important as the injection of 6-OHDA could  
401 induce serotonergic lesions, although we observed marked changes in the contralateral side of  
402 the brain, which would not be directly impacted by the toxin.

403 Finally, our results demonstrate that the coupling state of 5-HT<sub>1A</sub>R can be strongly disrupted in  
404 Parkinson's disease and LID, supporting the use of agonist radiotracers in future clinical PET  
405 studies to revisit the pathophysiological involvement of these receptors. Such studies would  
406 contribute to a better understanding of the role of 5-HT<sub>1A</sub>R in the psychiatric and motor  
407 symptoms in Parkinson's disease. Focusing on the active state of 5-HT<sub>1A</sub>R may also constitute

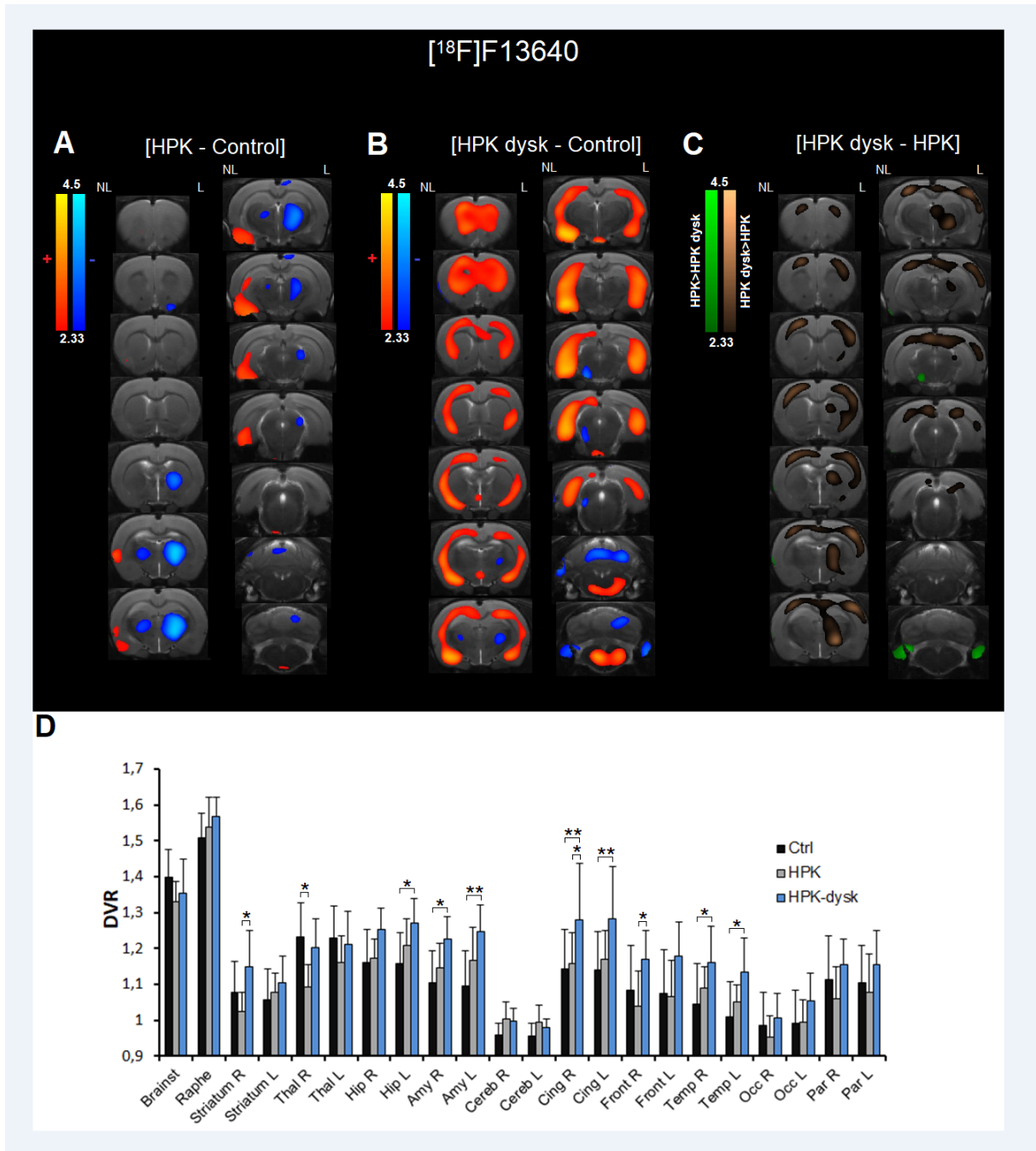
408 a biomarker for monitoring disease progression, or for measuring occupancy changes after  
409 administration of clinical doses of 5-HT<sub>1A</sub> agonists (5-HT<sub>1A</sub>R antagonist radiotracers being  
410 poorly sensitive to competition with agonists - see [90-94]). Overall, the use of agonist PET  
411 tracers opens multiple avenues of investigation for brain imaging of Parkinson's disease and  
412 other disorders involving serotonergic neurotransmission.

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420 **Legends**

421 **Figure 1: Effect of 6-OH-DA lesion and LID induction on [<sup>18</sup>F]MPPF PET imaging in rats.**

422 A - Voxel-to-voxel statistical comparisons of [<sup>18</sup>F]MPPF BP<sub>ND</sub> between control rats and  
423 hemiparkinsonian rats. Z scores in color scales (significant increases in red, significant  
424 decreases in blue; p<0.01). B - Voxel-to-voxel statistical comparisons of [<sup>18</sup>F]MPPF BP<sub>ND</sub>  
425 between control rats and hemiparkinsonian rats that developed LID. Z scores in color scales  
426 (significant increases in red, significant decreases in blue; p<0.01). C - Voxel-to-voxel  
427 statistical comparisons of [<sup>18</sup>F]MPPF BP<sub>ND</sub> between hemiparkinsonian rats that developed LID  
428 and untreated hemiparkinsonian rats. Z scores in color scales (higher BP<sub>ND</sub> in non-dyskinetic  
429 rats in green, higher BP<sub>ND</sub> in dyskinetic rats in brown; p<0.01). D - Mean DVR values (± SD)  
430 in the different regions of interest for each group (\*p<0.05, \*\*p<0.01).

431

432 **Figure 2: Effect of 6-OH-DA lesion and LID induction on [<sup>18</sup>F]F13640 PET imaging in**

433 **rats.** A - Voxel-to-voxel statistical comparisons of [<sup>18</sup>F]F13640 BP<sub>ND</sub> between control rats and  
434 hemiparkinsonian rats. Z scores in color scales (significant increases in red, significant  
435 decreases in blue; p<0.01). B - Voxel-to-voxel statistical comparisons of [<sup>18</sup>F]F13640 BP<sub>ND</sub>  
436 between control rats and hemiparkinsonian rats that developed LID. Z scores in color scales  
437 (significant increases in red, significant decreases in blue; p<0.01). C - Voxel-to-voxel  
438 statistical comparisons of [<sup>18</sup>F]F13640 BP<sub>ND</sub> between hemiparkinsonian rats that developed  
439 LID and untreated hemiparkinsonian rats. Z scores in color scales (higher BP<sub>ND</sub> in non-  
440 dyskinetic rats in green, higher BP<sub>ND</sub> in dyskinetic rats in brown; p<0.01). D - Mean DVR  
441 values (± SD) in the different regions of interest for each group (\*p<0.05, \*\*p<0.01).

442

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447 **Conflict of Interest**

448 Dr. Newman-Tancredi is an employee and stockholder of Neurolixis.

449 The other authors have no conflict of interest to report.

450

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