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Different alterations of agonist and antagonist binding to 5-HT_{1A} receptor in a rat model of Parkinson's disease and levodopa-induced dyskinesia : a microPET study

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Running title: 5-HT_{1A}R agonist and antagonist binding in Parkinson's disease and LID

Key words: 5-HT_{1A}; Parkinson's disease; serotonin; Levodopa-induced dyskinesia; GPCR

Abstract:

BACKGROUND : The gold-standard treatment for Parkinson's disease is L-DOPA, which in the long term often leads to levodopa-induced dyskinesia. Serotonergic neurons are partially responsible for this, by converting L-DOPA into dopamine before uncontrolled release as a "false neurotransmitter". The stimulation of 5-HT_{1A} receptors can reduce involuntary movements but this mechanism is poorly understood.

OBJECTIVES : This study aimed to investigate the functionality of 5-HT_{1A} receptor using positron emission tomography in hemiparkinsonian rats with or without dyskinesia induced by 3-weeks daily treatment with L-DOPA. Imaging sessions were performed "off" L-DOPA.

METHODS : Each rat underwent a positron emission tomography scan with [^{18}F]F13640, a 5-HT_{1A}R agonist (which labels receptors in a high affinity state for agonists) or [^{18}F]MPPF, a 5-HT_{1A}R antagonist (which labels all the receptors).

RESULTS: There were decreases of [^{18}F]MPPF binding in hemiparkinsonian rats in cortical areas. In dyskinetic animals, changes were slighter but also found in other regions. In hemiparkinsonian rats, [^{18}F]F13640 uptake was decreased in the globus pallidus and thalamus. On the contralateral side, binding was increased in the insula, the hippocampus and the amygdala. In dyskinetic animals, [^{18}F]F13640 binding was strongly increased in cortical and limbic areas, especially in the non-lesioned side.

CONCLUSION : These data suggest that agonist and antagonist 5-HT_{1A} receptor-binding sites are differently modified in Parkinson's disease and levodopa-induced dyskinesia. In particular, these observations suggest an important involvement of the functional state of 5-HT_{1A}R in levodopa-induced dyskinesia and emphasize the need to characterize this state using agonist radiotracers in physiological and pathological conditions.

Introduction

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

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

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

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

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

We recently developed and validated in animal the first fluorinated agonist radiotracer of 5-HT_{1A} receptors, [^{18}F]F13640 [34], and we have transferred it to humans as a new PET radiopharmaceutical [35]. This radiotracer is a full 5-HT_{1A}R agonist and displays a high affinity and selectivity for 5-HT_{1A} receptors [36, 37]

Here, the aim was to investigate changes of 5-HT_{1A}R coupling in the 6-OHDA-lesioned rat model of Parkinson's disease using both a 5-HT_{1A} antagonist radiotracer, [^{18}F]MPPF, and the

95 agonist radiotracer [^{18}]F13640. We also studied the functional state of 5-HT_{1A}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 ± 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 [^{18}F]MPPF and [^{18}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 [^{18}F]MPPF or [^{18}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®, 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 [^{18}F]MPPF (14.01 ± 2.30 MBq) or [^{18}F]F13640 (12.14 ± 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 ± 0.2, 2.7 ± 0.2, 2.5 ± 0.2 and 2.8 ± 0.2 (average
174 dyskinetic score ± 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_{1A} receptors using [^{18}F]MPPF*

178 [^{18}F]MPPF binding was compared between hemiparkinsonian rats with or without dyskinesia
179 and control rats (Fig. 1). At the voxel level, [^{18}F]MPPF BP_{ND} 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 [^{18}F]MPPF BP_{ND} 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 [^{18}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, [^{18}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. [^{18}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 [^{18}F]MPPF binding between HPK and HPK-LID rats (Fig. 1C). [^{18}F]MPPF
198 BP_{ND} 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, [^{18}F]MPPF BP_{ND} 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 [^{18}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_{1A} receptors in high-affinity state using [^{18}F]F13640*

206 Binding potentials of [^{18}F]F13640, reflecting changes in 5-HT_{1A} 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 [^{18}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 [^{18}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 [^{18}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 [^{18}F]F13640 binding between HPK and HPK-LID rats (Fig.
231 2C). [^{18}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 [^{18}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_{1A}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_{1A}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_{1A} 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_{1A}R may also play
250 a role in control of LID [17, 62]. There are also discrepancies regarding the effect of 5-HT_{1A}
251 agonists on motor symptoms of Parkinson's disease [63-65]. Few clinical studies have been
252 conducted with 5-HT_{1A} 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_{1A}
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_{1A}R levels and function.

258

The contribution of PET imaging in deciphering the involvement of 5-HT_{1A}R in Parkinson's disease

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

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

284 significantly correlated with the tremor score [24]. Another study using [^{18}F]MPPF showed
285 decreases of 5-HT_{1A}R binding in patients, depending on the comorbidity with depression [26].
286 A postmortem autoradiography study reported an increased binding of [^{3}H]WAY-100635 in
287 the temporal cortex of patients [21]. These data show a complex involvement of 5-HT_{1A}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_{1A}R: such tracers cannot characterize underlying changes in receptor-G-protein
296 coupling state. In contrast, the use of the innovative agonist radiotracer, [^{18}F]F13640, allowed
297 us for the first time to examine changes in active state 5-HT_{1A}R in the present model of
298 Parkinson's disease.

299

300 *PET imaging of high-affinity state 5-HT_{1A} 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_{1A}R in the active (i.e. G-protein-coupled) state, as determined by [^{18}F]F13640 binding, were
304 completely distinct from the changes in the whole population of receptors, as visualized using
305 [^{18}F]MPPF.

306 Using [^{18}F]MPPF, 5-HT_{1A}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 [^{18}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_{1A}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 [^{18}F]MPPF is poorly
314 sensitive to supraphysiological changes in serotonin concentration [34]. Therefore, cortical
315 decreases of [^{18}F]MPPF binding potential could be related to an altered affinity for receptors or
316 to a lower density of 5-HT_{1A} 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, [^{18}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_{1A}
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_{1A} 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 [^{18}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_{1A}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 [^{18}F]F13640 binding. Another
332 important consideration is that [^{18}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 [^{18}F]F13640 binding. As seen in the voxel analysis, [^{18}F]F13640 binding
335 modifications were spatially distinct from that of [^{18}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 [^{18}F]MPPF).
339 In HPK rats, [^{18}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_{1A}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_{1A} 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, [^{18}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 [^{18}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

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

384 of 5-HT_{1A}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 [^{18}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_{1A}R in the high-affinity state, suggesting a hypersensitivity of 5-HT_{1A}R in
396 the lesioned side of the striatum.

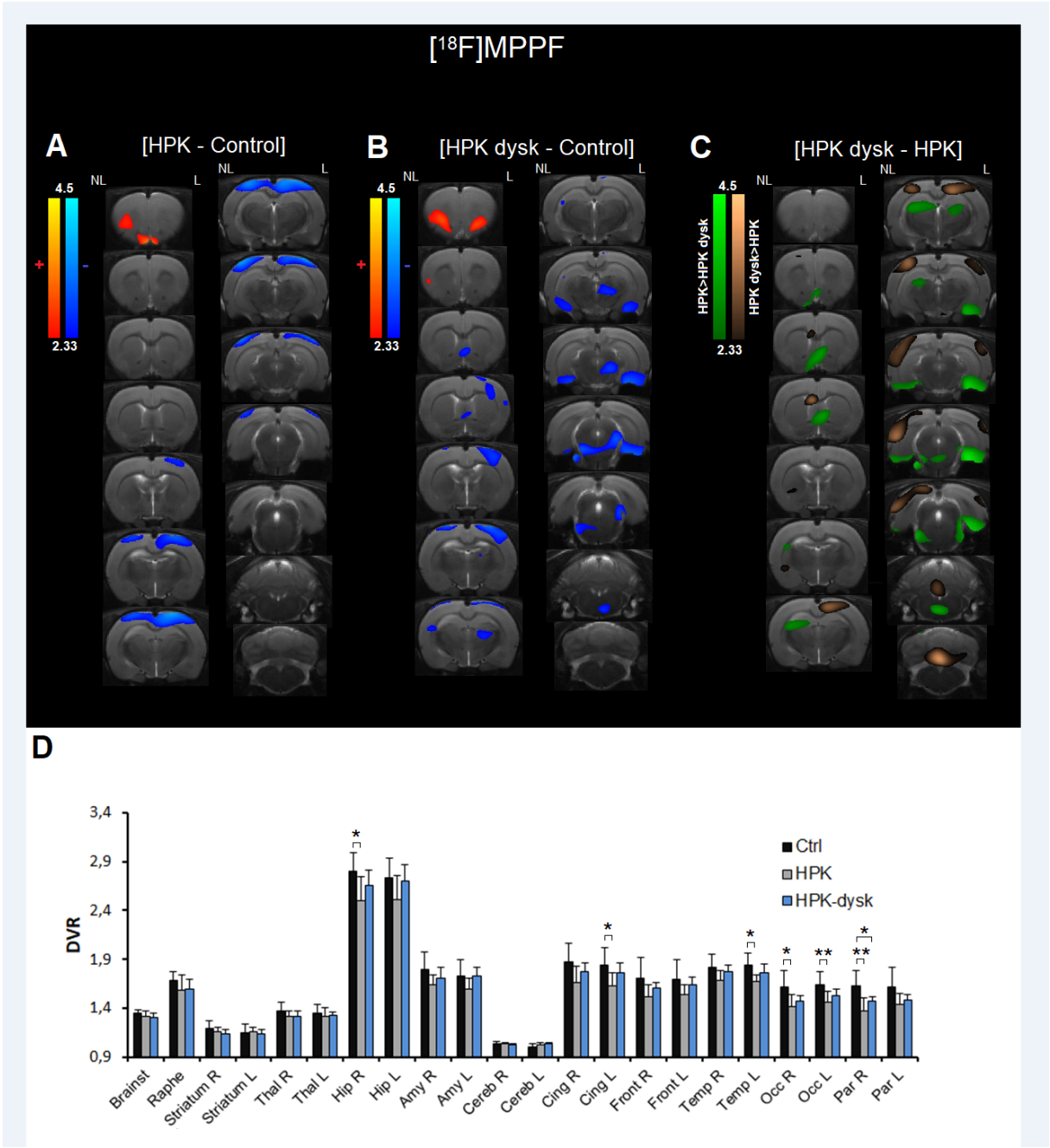
397 As previously discussed, changes occurring at the level of presynaptic serotonergic
398 transmission could explain some of our results with [^{18}F]F13640. In the future, it would be
399 valuable to evaluate the changes of [^{18}F]F13640 along with the extracellular 5-HT concentration
400 and the 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_{1A}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_{1A}R in the psychiatric and motor
407 symptoms in Parkinson's disease. Focusing on the active state of 5-HT_{1A}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_{1A} agonists (5-HT_{1A}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.

413

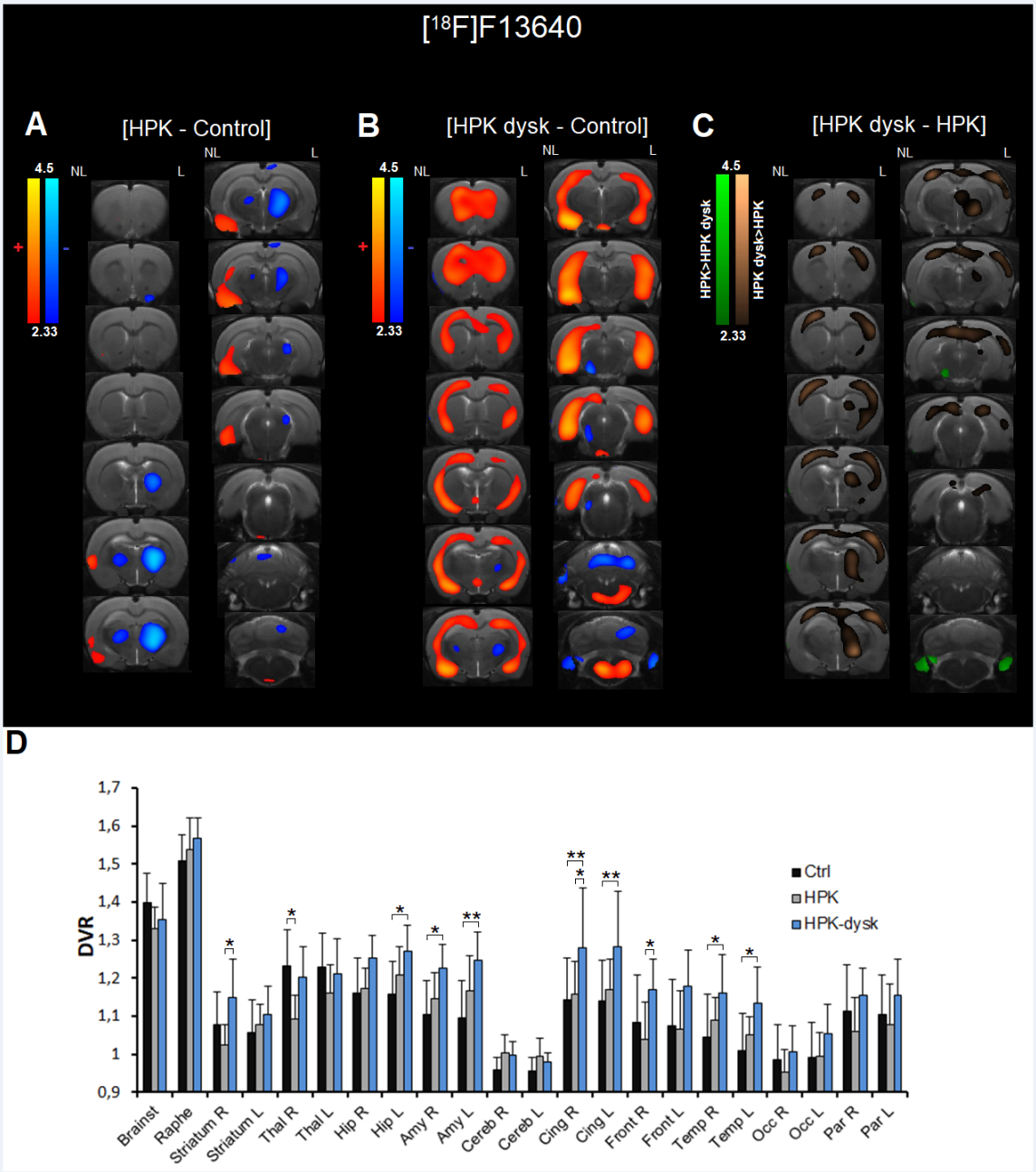
414 **Figure 1**



415

416

417 **Figure 2**



418

419

420 **Legends**

421 Figure 1: **Effect of 6-OH-DA lesion and LID induction on [^{18}F]MPPF PET imaging in rats.**
422 A - Voxel-to-voxel statistical comparisons of [^{18}F]MPPF BP_{ND} 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 [^{18}F]MPPF BP_{ND}
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 [^{18}F]MPPF BP_{ND} between hemiparkinsonian rats that developed LID
428 and untreated hemiparkinsonian rats. Z scores in color scales (higher BP_{ND} in non-dyskinetic
429 rats in green, higher BP_{ND} 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 [^{18}F]F13640 PET imaging in**
433 **rats.** A - Voxel-to-voxel statistical comparisons of [^{18}F]F13640 BP_{ND} 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 [^{18}F]F13640 BP_{ND}
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 [^{18}F]F13640 BP_{ND} between hemiparkinsonian rats that developed
439 LID and untreated hemiparkinsonian rats. Z scores in color scales (higher BP_{ND} in non-
440 dyskinetic rats in green, higher BP_{ND} 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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Conflict of Interest

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

The other authors have no conflict of interest to report.

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