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# Change in Expression of 5-HT<sub>6</sub> Receptor at Different Stages of Alzheimer's Disease: A Postmortem Study with the PET Radiopharmaceutical [<sup>18</sup>F]2FNQ1P

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## Abstract.

**Background:** The 5-HT<sub>6</sub> receptor is one of the most recently identified serotonin receptors in the central nervous system. Because of its role in memory and cognitive process, this receptor might be implicated in Alzheimer's disease (AD) and associated disorders.

**Objective:** The aim of this study was to investigate the binding of [<sup>18</sup>F]2FNQ1P, a new specific radiotracer of 5-HT<sub>6</sub> receptors, and to quantify 5-HT<sub>6</sub> receptor density in caudate nucleus in a population of patients with different AD stages.

**Methods:** Patients were classified according to the "ABC" NIA-AA classification. *In vitro* binding assays were performed in postmortem brain tissue from the healthy control (HC; *n* = 8) and severe AD ("High"; *n* = 8) groups. *In vitro* quantitative autoradiography was performed in human brain tissue (caudate nucleus) from patients with different stages of AD: HC (*n* = 15), "Low" (*n* = 18), "Int" (*n* = 20), and "High" (*n* = 15).

**Results:** *In vitro* binding assays did not show significant differences for the K<sub>D</sub> and B<sub>max</sub> parameters between "High" and HC groups. *In vitro* quantitative autoradiography showed a significant difference between the "High" and HC groups (*p* = 0.0025). We also showed a progressive diminution in [<sup>18</sup>F]2FNQ1P specific binding, which parallels 5-HT<sub>6</sub> receptors expression, according to increasing AD stage. Significant differences were observed between the HC group and all AD stages combined ("Low", "Intermediate", and "High") (*p* = 0.011).

**Conclusion:** This study confirms the interest of investigating the role of 5-HT<sub>6</sub> receptors in AD and related disorders. [<sup>18</sup>F]2FNQ1P demonstrated specific binding to 5-HT<sub>6</sub> receptors.

Keywords: 5-HT<sub>6</sub> receptor, Alzheimer's disease, caudate nucleus, [<sup>18</sup>F]2FNQ1P, specific PET radiotracer

## INTRODUCTION

Serotonin is reported to have many neurological functions as a neurotransmitter, in the central nervous system but also in many other organ systems:

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cardiovascular, pulmonary, and genitourinary [1]. Serotonin receptor studies identified 7 sub-families of serotonin receptor, with many subtypes in each [2]. The 5-HT<sub>6</sub> receptor is one of the most recently identified, first in rat [3,4] and then in the human brain [5]. The 5-HT receptor family is involved in many physiological processes such as memory, learning, and food intake. Many studies also demonstrated roles in pathophysiological processes, including Alzheimer's disease (AD) [6–15].

The pathophysiology of AD involves several mechanisms, from formation of extracellular amyloid- $\beta$  plaques [16], to neurofibrillary tangles in the intracellular environment [17], and consecutive neuronal death and synapse loss, all leading to progressive cognitive decline [18]. While the molecule-candidates targeting aggregated proteins have so far been therapeutic failures, a new strategy has emerged in parallel, seeking to target neurotransmission systems more specifically, with the objective of improving the clinical semiology of cognitive decline. An immunohistochemistry study revealed a significant reduction in 5-HT<sub>6</sub> receptor density in cortical areas of AD patients [19], opening up the possibility of specific targeting of this receptor at an early stage of the disease. Several clinical studies evaluated 5-HT<sub>6</sub> receptor antagonists in phase I, II, or III clinical trials for the treatment of AD. Early results were mixed: a phase I study showed the antagonist PRX-07034 to be selective for 5-HT<sub>6</sub>, improving short-term memory, while a phase II study reported no benefit of another antagonist (SAM-760) on measures of cognition and neuropsychiatric symptoms in AD patients [20]. Unfortunately, another phase III clinical trial failed, testing idalopirdine, a 5-HT<sub>6</sub> receptor antagonist originally developed for cognitive improvement in AD [21]. However, other 5-HT<sub>6</sub> receptor antagonist compounds are still being investigated [22], such as SUVN-502, currently in phase II trial [23]. Furthermore, 5-HT<sub>6</sub> receptor agonists have paradoxically also been shown to have cognitive enhancement properties [10], leaving this target in the race for symptomatic treatment of AD. Nevertheless, it has to be recognized that research concerning antagonists are more developed than agonists.

In this context, visualizing and quantifying 5-HT<sub>6</sub> receptors during the patient's lifetime through PET imaging would be a valuable contribution to understanding this therapeutic target. The development of a specific radiotracer for 5-HT<sub>6</sub> receptors seems necessary for better understanding of 5-HT<sub>6</sub> receptor

mechanisms in AD, classification of AD grades and long-term follow-up of patients treated with 5-HT<sub>6</sub> antagonists. Several radiotracers have been described in literature, but none stands out as specific for the 5-HT<sub>6</sub> receptor [24, 25]. Our laboratory recently developed a new specific radiotracer for these receptors, [<sup>18</sup>F]2FNQ1P. A previous study described the synthesis of its precursor and its radiolabeling [26]. A recent pre-clinical study in non-human primates suggested that [<sup>18</sup>F]2FNQ1P is a reliable PET radiotracer for visualization and quantification of 5-HT<sub>6</sub> receptors [27].

Regional distribution and associated neuronal or glial expression of 5-HT<sub>6</sub> receptors were investigated on human postmortem tissue by both autoradiography with [<sup>125</sup>I]SB258585 and immunohistochemistry [28, 29]. These studies revealed high levels in the striatum, moderate levels in the hippocampus and cerebral cortex, and low levels in cerebellum.

The aim of the present study was to investigate postmortem binding of [<sup>18</sup>F]2FNQ1P in the caudate nucleus in a population of patients at different AD stages. This region, rarely explored in the context of AD [16], was chosen because of its high density of 5-HT<sub>6</sub> receptors, favorable for quantitative autoradiographic analyses. We hypothesized that 5-HT<sub>6</sub> receptor density would correlate with the stage of AD progression and severity.

## MATERIALS AND METHODS

### *Subjects and tissue samples*

Adjacent unstained frozen slices (30  $\mu$ m thickness) from human caudate nuclei ( $n=68$ ) were obtained from the London Neurodegenerative Diseases Brain Bank [30]. Sixteen fresh samples of caudate nucleus were also obtained: 10 from the 68 patients of the Medical Research Council (London) Neurodegenerative Diseases Brain Bank and 6 from the Medical Research Council (Lyon) bank (CardioBioTec, Lyon Hospitals) after approval by the hospital department review board. Patient ages, postmortem interval, and sex ratio were not significantly different ( $p>0.05$ ) between patient groups (Table 1). Tissues were collected through appropriate consent procedures under the ethics procedures of the brain banks. Patients were stratified according to the guidelines of the National Institute on Aging-Alzheimer's Association [31] This classification uses an "ABC" staging protocol for the

Table 1  
Demographic data of patients

AD Stages	HC	Low	Int	High
Number of cases	15	18	20	15
Age (y)	83.4 ± 4.5	86 ± 4.1	87.3 ± 2.9	83.1 ± 4.2
Gender (M/F)	6/9	7/12	15/5	9/6
Postmortem interval (h)	47.1 ± 13.7	47.8 ± 8.9	51.1 ± 10.4	46.2 ± 13.9

No significant differences were observed between groups for age, sex ratio, and postmortem interval criteria ( $p > 0.05$ ). HC, healthy control.

neuropathologic changes in AD, based on three morphologic characteristics of the disease: amyloid- $\beta$  plaques (A), neurofibrillary tangles (B), and neuritic plaques (C). Using a system of scores for each group, the ABC score was transformed into four levels of AD neuropathologic alteration: HC (healthy control group), “Low”, “Int” (intermediate), or “High”.

For unstained frozen slices, 15 patients were in the “High” stage of AD, 20 “Int”, 18 “Low”, and 15 HC. For fresh caudate tissues, 8 patients were HC and 8 “High”. The tissues were stored at  $-80^{\circ}\text{C}$  and defrosted extemporaneously.

#### Radiosynthesis of the 5-HT<sub>6</sub> receptor radiopharmaceutical and quality controls

The chemical nitro-precursor of our 5-HT<sub>6</sub> PET radiotracer, [<sup>18</sup>F]2FNQ1P, was synthesized as described previously [26]. Radiolabeling with <sup>18</sup>F was performed extemporaneously, on the days of the *in vitro* experiments, according to our published protocol [32]. Briefly, radiosynthesis used an automated Neptis fluorination module (OOC, Belgium). [<sup>18</sup>F]2FNQ1P quality control determined radiochemical purity and molar activity on analytical HPLC assay at the end of each production run, guaranteeing the radiopharmaceutical quality of the radiotracer used for the following *in vitro* experiments: i.e., molar activity  $> 340$  GBq/ $\mu\text{mol}$  and radiochemical purity  $> 98\%$ .

#### *In vitro* binding assays

*In vitro* binding assays were performed in post-mortem fresh caudate nucleus samples from the HC and “High” groups. Briefly, tissues were preserved in phosphate buffered saline (PBS)/EDTA 0.1% and ground with buffer (50 mM Tris-HCl pH 7.4 at  $25^{\circ}\text{C}$ ) (BetaPrion, BSE Purification kit, HMOGEN TUB, biochemistry). Homogenates were centrifuged for 20 min at 35,000 g (Discovery M150 SE ultra-

centrifuge, Hitachi). The pellet was resuspended in 50 mM Tris-HCl (pH 7.4 at  $25^{\circ}\text{C}$ ) and incubated for 15 min at  $37^{\circ}\text{C}$ . Following two further centrifugation steps (as above), the membranes were finally resuspended and stored at  $-80^{\circ}\text{C}$  until use.

Brain tissues were preserved in buffer containing 50 mM Tris-HCl, 10  $\mu\text{M}$  pargyline, 5 mM MgCl<sub>2</sub>, 5 mM ascorbate, and 0.5 mM EDTA (pH 7.4). Binding assay consisted of 50  $\mu\text{L}$  displacing compound (SB-258585 1  $\mu\text{M}$ : a 5-HT<sub>6</sub> receptor antagonist) or buffer, 100  $\mu\text{L}$  membrane suspension (corresponding to approximately 60  $\mu\text{g}$  protein per well for brain tissue) and 50  $\mu\text{L}$  [<sup>18</sup>F]2FNQ1P (molar activity, 59.2 GBq/ $\mu\text{mol}$ ). [<sup>18</sup>F]2FNQ1P was used at concentrations from 0.05 to 10 nM. Association rates were determined by incubation of membranes with radioligand at  $25^{\circ}\text{C}$  for 60 min before termination of the experiment. Bound radiolabeled tracer was separated from free tracer by filtration under reduced pressure (MultiScreen HTS-FB, Millipore). Filters were washed 6 times with 200  $\mu\text{L}$  PBS. Washed filters were assayed for radioactivity by  $\gamma$ -counter (Gamma Wizard 2480, Perkin Elmer).

#### Quantitative *in vitro* 5-HT<sub>6</sub> receptor autoradiography

*In vitro* autoradiography was performed on adjacent unstained frozen slices of human brain tissue (caudate nucleus) from patients with different stages of AD. For each patient, pairs of adjacent slices were defrosted. The first slide was incubated for 60 min in buffer containing 50 mM Tris-HCl, 10  $\mu\text{M}$  pargyline, 5 mM MgCl<sub>2</sub>, 5 mM ascorbate, and 0.5 mM EDTA (pH 7.4) and 18.5 kBq/mL [<sup>18</sup>F]2FNQ1P, to measure total binding, and the second was incubated in the same buffer with SB-258585 at a concentration of 100 nM, for the competition experiment to measure non-specific binding. Each slide contained two adjacent of caudate nucleus sections constituting duplicate for total and specific binding. After incubation, the slices were washed in distilled water,

214 then dried and placed on a phosphor imaging plate  
 215 (BAS-5000, Fujifilm) for 60 min. The films were ana-  
 216 lyzed by a computer-assisted image analysis system  
 217 (Multigauge, Fujifilm). For each patient, a region-  
 218 of-interest (ROI) was manually drawn on one of the  
 219 two sections of the slide to measure the total binding.  
 220 This ROI was replicated on the other three sections  
 221 (total binding and non-specific binding) to compare  
 222 regions with a same size. These ROIs were positioned  
 223 in homogeneous areas, avoiding folds or tissue dam-  
 224 age due to poor sample quality, if any. The mean of  
 225 total binding values and non-specific binding values  
 226 (in PSM/mm<sup>2</sup>) was calculated for each slide. Specific  
 227 binding was determined by subtracting non-specific  
 228 binding from total binding values.

229 In parallel, calibration standards were prepared  
 230 from rat brain tissue homogenates, as described in  
 231 a previous study [33]. Briefly, crushed rat brains  
 232 were homogenized, and proteins were quantified by  
 233 a chemical analyzer. The rest of the homogenates  
 234 was frozen at -80°C. For each radiosynthesis, four  
 235 homogenates were defrosted and mixed with increas-  
 236 ing radioligand activities (116, 231, 463, and 925  
 237 kBq). The mixture was then refrozen in isopentane

238 cooled with dry ice. The frozen samples were then  
 239 cut into 30µm coronal slices using a -20°C cryostat.  
 240 The slices were placed on the human brain slices on  
 241 the imaging plates and signal-to-concentration curves  
 242 were calculated.

#### 243 Data analysis

244 ANOVA tests assessed group homogeneity for  
 245 age, gender, and postmortem caudate tissue sampling  
 246 interval (Table 1).

247 In radioligand binding studies,  $K_D$  and  $B_{max}$  val-  
 248 ues were calculated using GraphPad Prism software  
 249 (Graph Pad Software, Prism 6). Statistically signif-  
 250 icant variations in radioligand binding parameters  
 251 were assessed on non-parametric Mann-Whitney test.  
 252 For the quantitative autoradiography study, ANOVA  
 253 assessed statistical differences. In case of a differ-  
 254 ence, results were compared between groups with  
 255 on *post-hoc* Bonferroni correction test. Correlations  
 256 between AD stages (quantified as: HC = 0; Low = 1;  
 257 Int = 2; High = 3) and specific binding were assessed  
 258 on Pearson's *r*. For all analyses, the significance  
 259 threshold was set at  $p < 0.05$ .

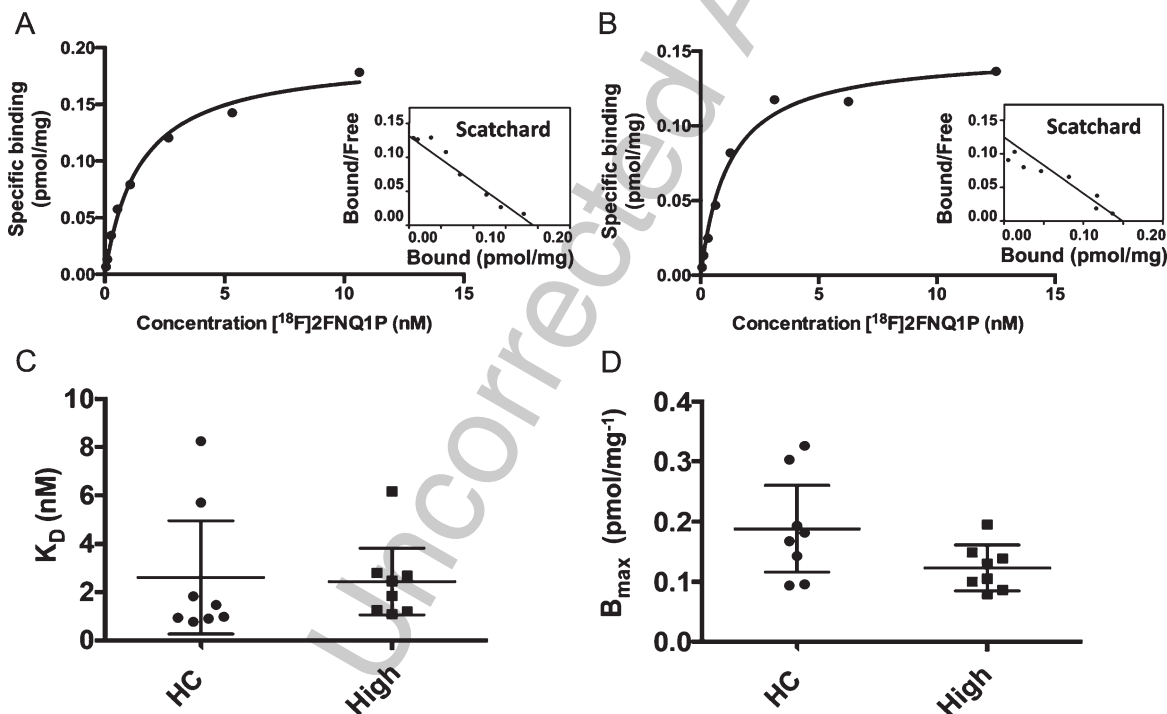


Fig. 1. *In vitro* binding assays. Example of saturation binding curves and Scatchard plots with fresh caudate nucleus samples from HC (A) and “High” AD stage (B) using the 5-HT<sub>6</sub> radiopharmaceutical [<sup>18</sup>F]2FNQ1P. No significant differences were observed between HC and “High” groups for the dissociation constant ( $K_D$ ) (C) or number of binding sites ( $B_{max}$ ) (D; Mann-Whitney test,  $p > 0.05$ ). Bars plot mean and 95% confidence interval.

## RESULTS

### *In vitro* binding assays

*In vitro* binding assay showed reproducible binding of [<sup>18</sup>F]2FNQ1P to 5-HT<sub>6</sub> receptors in the HC and “High” groups (Fig. 1A, B). Mean radioligand equilibrium dissociation constants ( $K_D$ ) were  $2.6 \pm 2.8$  nM and  $2.4 \pm 1.7$  nM the HC group and “High” groups, respectively (Fig. 1C). Total 5-HT<sub>6</sub> receptor density ( $B_{max}$ ) was  $0.19 \pm 0.09$  and  $0.12 \pm 0.04$  for the HC group and “High” groups, respectively, the difference being non-significant (Fig. 1D).

### *In vitro* quantitative 5-HT<sub>6</sub> receptor autoradiography

Regardless AD stage, all samples showed binding for [<sup>18</sup>F]2FNQ1P according to the location of the 5-HT<sub>6</sub> receptor in the caudate nucleus. Figure 2A represents an example of the total and non-specific binding of the [<sup>18</sup>F]2FNQ1P depending on the stage of AD. Mean percentage specific binding compared to total binding was respectively  $21.1 \pm 9.7$ ,  $16.9 \pm 15.3$ ,  $16.5 \pm 12.6$ , and  $10.9 \pm 15.4$  for HC group, “Low”, “Int”, and “High” stages. Results are presented in Fig. 2 according to AD stage. Mean specific binding was respectively  $96.1 \pm 27.6$ ,  $68.4 \pm 41.4$ ,  $70.9 \pm 48.7$ , and  $39.3 \pm 43.5$  pmol/mg of protein for HC group, “Low”, “Int”, and “High” stages. ANOVA showed a significant difference between groups ( $p = 0.004$ ). *Post-hoc* Bonferroni correction revealed a significant difference between the “High” and HC groups ( $p = 0.0025$ ) (Fig. 2B). *Post-hoc* tests did not reveal significant differences between HC, “Low”, and “Int” groups, but specific binding showed a strong negative correlation with all stages from HC to “High” ( $r = -0.42$ ,  $p < 0.01$ ). A significant difference ( $p = 0.011$ ) was also found between HC group and all AD stages combined (“Low”, “Int”, and “High”) on *post-hoc* *t*-test with Bonferroni correction (Fig. 2C).

## DISCUSSION

To our knowledge this is the first study investigating a specific PET radiotracer of 5-HT<sub>6</sub> receptors in a population of AD patients with different stages of disease. The results showed a decrease in caudate 5-HT<sub>6</sub> receptor density with [<sup>18</sup>F]2FNQ1P in subjects with AD compared to control group.

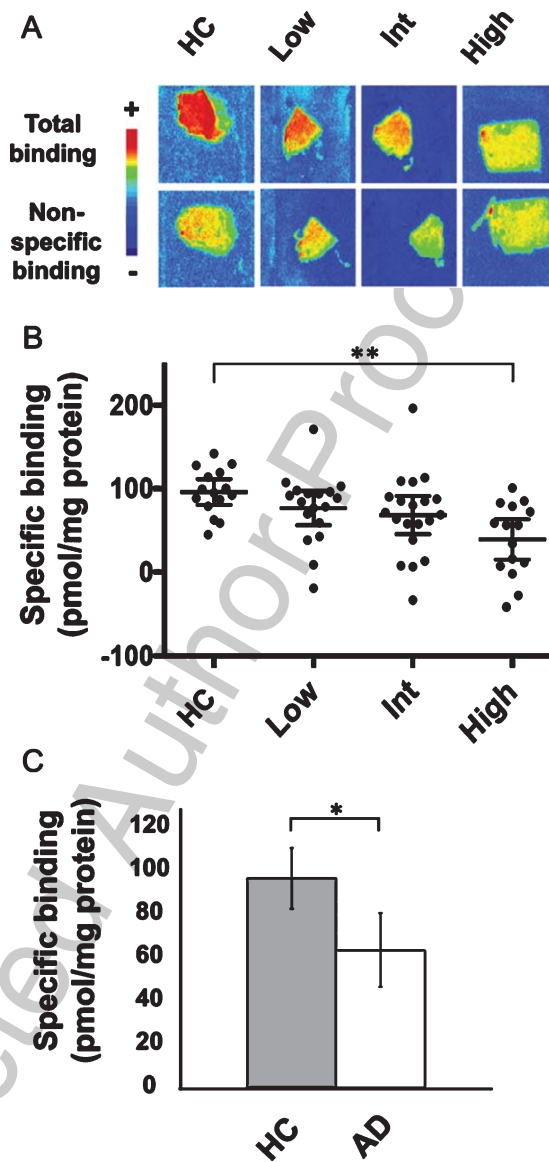


Fig. 2. Autoradiographic quantification of specific binding of the 5-HT<sub>6</sub> radiopharmaceutical [<sup>18</sup>F]2FNQ1P according to group of patients. A) Example of regional distribution of [<sup>18</sup>F]2FNQ1P binding sites in the caudate nucleus in HC and patient groups. The autoradiographs showing the decrease of [<sup>18</sup>F]2FNQ1P binding when SB258585 is added during incubation represent non-specific binding. B) A significant difference was seen between the “High” and HC groups (\*\* $p < 0.01$ ). Bars plot mean and 95% confidence interval. C) A significant difference was also found between HC group and all AD stages combined (“Low”, “Int” and “High”) on *post-hoc* *t*-test with Bonferroni correction (\* $p < 0.05$ ). Error-bars with 95% confidence intervals of the means do not overlap.

As previously outlined, [<sup>18</sup>F]2FNQ1P is the only PET radiopharmaceutical currently available that is specific for 5-HT<sub>6</sub> receptors [32]. In addition, [<sup>18</sup>F]2FNQ1P has a high specificity for 5-HT<sub>6</sub>

receptors with comparison to the following serotonergic receptors, 5-HT<sub>2A</sub>, 5-HT<sub>2B</sub>, 5-HT<sub>2C</sub>, 5-HT<sub>1B</sub>, 5-HT<sub>4</sub>, as well as for dopamine (D<sub>2</sub>, D<sub>3</sub>) and muscarinic ( $\alpha_{1B}$ ) receptors, which are very present in the striatal regions [26]. In this study, binding assays showed reliable affinity of [<sup>18</sup>F]2FNQ1P for these receptors. Radioligand saturation binding assays found no significant difference between the HC and “High” groups on the K<sub>D</sub> and the B<sub>max</sub> parameters. The high affinity of [<sup>18</sup>F]2FNQ1P for 5-HT<sub>6</sub> was confirmed by the mean K<sub>D</sub> for each group (around 2.5 nM). This result is consistent with a previous study which found a K<sub>D</sub> of approximately 1 nM [26]. Although B<sub>max</sub> was lower in the “High” than the HC group, the difference was non-significant. These results could be explained by the small size of the study population (*n* = 8). This explanation is frequently put forward in postmortem studies, as access to brains of neuropathologically documented AD subjects is limited. However, further investigations with a larger sample might demonstrate a significant difference, such as was found in the autoradiography assay.

Quantitative autoradiography experiments showed that [<sup>18</sup>F]2FNQ1P was suitable for visualizing and quantifying striatal 5-HT<sub>6</sub> receptor density. *In vitro* competition assays with the 5-HT<sub>6</sub> antagonist SB-258585 showed displacement of the radiotracer, confirming binding reversibility. Quantification of 5-HT<sub>6</sub> receptors revealed significant differences between the healthy control group (i.e., patients without AD neuropathological modifications) and patients with a high level of neuropathological modification (“High”). The results also distinguished the healthy control group from all AD stages. On the other hand, no significant differences were observed between the HC or “High” groups and other stages of AD (“Low” and “Int”). Despite these non-significant differences, the results showed a progressive decrease in the 5-HT<sub>6</sub> receptor expression according to AD stage, with a strong negative correlation between specific binding of [<sup>18</sup>F]2FNQ1P and disease stage. Here again, the absence of significant difference between the “High” group and earlier stages of AD can be initially explained by the relatively low number of patients in each group. Larger groups would reinforce the power of the study and could demonstrate significant differences. Another explanation concerns the complexity of assessing AD. Because of the large inter-individual variability found in AD, classification can be difficult. This is why many classifications exist, drawn up by different working groups such as

the IWG-2 classification of the International Working Group for New Research Criteria for the Diagnosis of Alzheimer’s Disease [34] and, more recently, the “ABC” classification of the National Institute of Aging-Alzheimer’s Association (NIA-AA) [31], used in the present study. This classification considers AD as a continuum, needing to be diagnosed in its early stages. However, these criteria remain to be validated. Lowe et al. applied this classification to the Alzheimer Disease Neuroimaging Initiative (ADNI) cohort [35]. The study showed the weakness of the NIA-AA criteria because of the complexity of interpreting biomarkers. Other studies assessing the applicability of this classification in AD patients with mild cognitive impairment showed the same weakness: variability in biomarker interpretation, lack of measurement standardization, and varying results [36, 37]. Furthermore, the “ABC” staging is strongly nested in other diseases contributing to cognitive impairment, such as Lewy body disease, vascular brain injury, or hippocampal sclerosis [38]. Thus, patient groups are difficult to distinguish, making it difficult to show significant differences between neighboring groups.

In addition, “ABC” staging is mostly based on anatomopathological criteria. The classification takes account of onset of A $\beta$  or amyloid plaques, neurofibrillary tangles, and neuritic plaques, but not of clinical status. However, it is well known that anatomopathological modifications in AD correlate with disease symptomatology. Symptom onset correlates with brain atrophy [39], hypometabolism [40], and neurofibrillary changes [41]. Thus, the correlation between decreased 5-HT<sub>6</sub> receptor density and decrease in symptomatology could be an approach worth considering in future studies.

Another point to be taken into account in interpreting the present results concerns the polymorphism of the 5-HT<sub>6</sub> receptor. The human 5-HT<sub>6</sub> receptor protein is coded by chromosome 1 and the specific gene of the receptor contains many trinucleotide polymorphisms [5]. Some previous studies, in Chinese and Korean populations, showed an association between this polymorphism and AD, and considered this allele to be a risk factor [42, 43]. Another study, in a Caucasian population, found no significant differences between controls and AD patients [44], and considered this 5-HT<sub>6</sub> receptor polymorphism as a silent mutation that does not affect the function of the protein. To date, an association between 5-HT<sub>6</sub> polymorphism in AD patients and the binding properties of [<sup>18</sup>F]2FNQ1P is still an open question.

413 Although the present study is the first to visualize  
414 5-HT<sub>6</sub> receptors in AD patients with a radiophar-  
415 maceutical usable for neuroimaging *in vivo*, a few  
416 previous studies also showed a decrease in these  
417 receptors in AD and related disorders, using ded-  
418 icated *in vitro* exploration probes. Lorke et al.,  
419 in an immunohistochemistry study [45], reported  
420 decreased cellular expression of 5-HT<sub>6</sub> receptors  
421 in the prefrontal cortex of AD patients in com-  
422 parison with normal age-matched subjects. 5-HT<sub>6</sub>  
423 receptors were expressed in pyramidal cells and  
424 stellate-shaped cells, and AD patients showed a  
425 significant 40% decrease in 5-HT<sub>6</sub> receptor expres-  
426 sion. Garcia-Alloza et al. reported similar results.  
427 They assessed the involvement of serotonergic dis-  
428 turbance of 5-HT<sub>6</sub> receptors in AD impairment.  
429 Binding assays with [<sup>125</sup>I]SB-258585, an *in vitro*  
430 radiotracer, were performed on tissue samples from  
431 frontal and temporal cortex. Results showed a sig-  
432 nificant decrease in 5-HT<sub>6</sub> receptor density in both  
433 frontal (56% reduction) and temporal (58% reduc-  
434 tion) cortex in AD patients compared to controls [19].  
435 While these results were consistent with 5-HT<sub>6</sub> recep-  
436 tor involvement in AD, the authors did not use the  
437 clinicopathologic diagnostic classification used in the  
438 present study.

439 The present study also differs from a majority of  
440 previous studies in the choice of the brain region  
441 of interest: the caudate nucleus, well-known to be  
442 a region rich in 5-HT<sub>6</sub> receptors [46, 47]. It is known  
443 that 5-HT<sub>6</sub> receptors are highly and homogeneously  
444 concentrated in the caudate nucleus, putamen, and  
445 nucleus accumbens [29]. The first preclinical explo-  
446 rations of our radiopharmaceutical confirmed its  
447 preferential striatal binding [26, 27, 32], justifying  
448 the choice of this region for the present postmortem  
449 study. However, it must be recognized that the patho-  
450 physiological involvement of the caudate nucleus in  
451 AD is not yet well established. Brain atrophy and  
452 neuron loss occurs mainly in the frontal cortex, hip-  
453 pocampus, and limbic areas [48]. De Jong et al.  
454 suggested a distinction in striatal pattern morphology  
455 in AD patients compared to subjects with memory  
456 complaints without objective cognitive impairment  
457 [49]. Their study showed that cognitive impairment  
458 is related to the degree of surface deformity, hypothesiz-  
459 ing that associative and limbic cerebral networks are  
460 primarily affected in AD. These findings highlight the  
461 interest of tracing the progression of 5-HT<sub>6</sub> receptors  
462 as striatal marker during neurodegeneration.

463 This is all the more interesting as the 5-HT<sub>6</sub> recep-  
464 tor is also a potential therapeutic target in AD [50].

465 Recently, drug-candidates have been developed on  
466 the pharmacological hypothesis that 5-HT<sub>6</sub> recep-  
467 tor blockade induces acetylcholine release and so  
468 improves cognition processes in AD [51]. So far,  
469 clinical studies were disappointing. Idalopirdine, a  
470 5-HT<sub>6</sub> receptor antagonist, did not improve cogni-  
471 tion in patients with mild to moderate AD compared  
472 to placebo [21]; intepirdine, another selective 5-HT<sub>6</sub>  
473 receptor antagonist, did not show efficacy in a phase  
474 II study [52]. However, these early studies do not  
475 signify the abandonment of therapeutic targeting of  
476 5-HT<sub>6</sub> receptors. Their main limitations were the lack  
477 of knowledge of 5-HT<sub>6</sub> receptor status in groups of  
478 heterogeneous AD patients. The difficulty of stag-  
479 ing patients may have led to underdosing in phase  
480 III compared to phase II [53]. It is therefore crucial  
481 to have better knowledge of the progression of  
482 5-HT<sub>6</sub> receptors during the course of AD, through  
483 PET imaging, to highlight potential early biomark-  
484 ers, to refine the recruitment for future therapeutic  
485 trials, and, finally, to contribute to the understanding  
486 of 5-HT<sub>6</sub> drug-candidates by drug occupancy stud-  
487 ies. The translational results we obtained with the  
488 radiopharmaceutical [<sup>18</sup>F]2FNQ1P therefore encour-  
489 age us to implement a PET neuroimaging study in AD  
490 patients. Indeed, this *in vivo* exploration will enable  
491 us to quantify other brain regions expressing signif-  
492 icant densities of 5-HT<sub>6</sub> receptors relevant to AD,  
493 such as the entorhinal, parietal, or frontal cortex, and  
494 to explore the modification of their expression during  
495 the disease progression.

## 496 CONCLUSION

497 This *in vitro* study is the first to demonstrate a  
498 decrease in caudate 5-HT<sub>6</sub> receptor density with  
499 [<sup>18</sup>F]2FNQ1P, a PET radiopharmaceutical, in sub-  
500 jects with AD. The challenge will now be to transfer  
501 [<sup>18</sup>F]2FNQ1P to *in vivo* PET neuroimaging stud-  
502 ies to confirm an early decline in 5-HT<sub>6</sub> receptor  
503 expression during disease progression and provide  
504 better understanding of the pharmacology of future  
505 candidate molecules targeting this serotonergic  
506 receptor.

## 507 ETHICS APPROVAL AND CONSENT TO 508 PARTICIPATE

509 Tissues were collected through appropriate con-  
510 sent procedures under the ethics procedures of the  
511 brain banks. Previous consent to do experiments



was given at the time of brain donation, and no supplementary consent was needed for this study. The study was conducted according to the principles of the Helsinki and subsequent revisions.

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## AVAILABILITY OF DATA AND MATERIALS

The data sets supporting the conclusions of this article can be made available upon request through the corresponding author.

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