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[¹⁸F]RS-127445 radiosynthesis and evaluation as a 5-HT_{2B} receptor PET radiotracer in rat brain

Violette Richin^{a,b}, Caroline Bouillot^c, Sandrine Bouvard^a, Pierre Courault^{a,c,d},
Sophie Lancelot^{a,c,d}, Luc Zimmer^{a,c,d}, Wael Zeinyeh^{a,c,*}

^a Université Claude Bernard Lyon 1, Centre de Recherche en Neurosciences de Lyon, CNRS UMR5292, INSERM U1028, Lyon, France

^b Neurolix SAS, Castres, France

^c CERMEP-Imagerie du Vivant, Bron, France

^d Hospices Civils de Lyon, Lyon, France

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ABSTRACT

Serotonin (5-hydroxytryptamine, 5-HT) is a neurotransmitter involved in many physiological and pathological mechanisms through its numerous receptors. Among these, the 5-HT_{2B} receptor is known to play a key role in multiple brain disorders but remains poorly understood. Positron emission tomography (PET) can contribute to a better understanding of pathophysiological mechanisms regulated by the 5-HT_{2B} receptor. To develop the first PET radiotracer for the 5-HT_{2B} receptor, RS-127445, a well-known 5-HT_{2B} receptor antagonist, was labeled with fluorine-18. [¹⁸F]RS-127445 was synthesized in a high radiochemical purity and with a good molar activity and radiochemical yield. Preliminary PET scans in rats showed good brain penetration of [¹⁸F]RS-127445. However, competition experiments and *in vitro* autoradiography showed high non-specific binding, especially to brain white matter.

Serotonin receptors play a role in numerous neuropsychiatric disorders, making them promising targets for diagnostic and therapeutic purposes.¹ PET (positron emission tomography) brain imaging is a widely used technique for studying serotonin receptors in the brain. It enables the visualization and quantification of pharmacological targets leading to a better understanding of the pathophysiological mechanisms regulated by brain receptors. PET radiotracers for specific serotonergic receptors can also constitute useful tools for diagnostic purposes and can aid in preclinical and clinical pharmacological studies of drug candidates.² Many serotonergic receptors have been studied using PET imaging with specific radiotracers to this date. However, there is currently no specific radiotracer for the 5-HT_{2B} receptor.^{1,3}

The 5-HT_{2B} receptor, a G-protein-coupled receptor belonging to the serotonin receptor family, was originally discovered in the gastric fundus of rats.⁴ However, further research revealed its presence in the heart, liver, kidney, gastrointestinal smooth muscle cells and other organs in animals and humans.⁴⁻¹¹ Several studies have demonstrated that 5-HT_{2B} receptors are involved in the control of cell proliferation and differentiation, as well as in the regulation of gastrointestinal, vascular, cardiac, and pulmonary functions.¹² Activation of 5-HT_{2B} receptors has

been implicated in the pathogenesis of drug-induced valvular heart disease (VHD).¹³ For this reason, benfluorex (Mediator®), an anorexic and antidiabetic molecule which acts as a 5-HT_{2B} receptor activator, has been withdrawn from the market.¹⁴ Similarly, the use of the antiparkinsonian drugs cabergoline and pergolide has been shown to be associated with VHD *via* an off-target effect by activating 5-HT_{2B} receptors.¹⁵ Recently, there has been renewed interest in 5-HT_{2B} receptors due to the emergence of psychedelics as potential new treatments for many psychiatric disorders. Most of the psychedelics have agonist activity for 5-HT_{2B} receptors, which could lead to VHD.¹⁶

The 5-HT_{2B} receptor is also present in the central nervous system (CNS) and plays a role in the regulation of several pathways. It is expressed in serotonergic, GABAergic, and dopaminergic neurons of rats and mice, particularly in the dorsal raphe nucleus (DRN).^{17,18}

In addition, immunohistochemical studies examining 5-HT_{2B} receptor protein expression in the rat brain have shown its presence in the frontal cortex (on the neuronal fibers but not cell bodies), cerebellum (Purkinje cells), lateral septum, dorsal hypothalamus and medial amygdala.¹⁹ The 5-HT_{2B} receptor is also found to be highly expressed in astrocytes²⁰ and microglia.²¹

* Corresponding author at: CERMEP-Imagerie du Vivant, 59 bd Pinel, 69677 Bron, France.

E-mail address: wael.zeinyeh@univ-lyon1.fr (W. Zeinyeh).

Targeting the 5-HT_{2B} receptor in the brain holds therapeutic promises. Indeed, it is involved in the regulation of dopaminergic neurotransmission, and 5-HT_{2B} antagonists have been found to improve certain symptoms of schizophrenia.²² In addition, 5-HT_{2B} receptor blockade inhibits MDMA-, amphetamine- and cocaine-induced hyperlocomotion in animal models.^{17,23,24}

Within the serotonergic system, several studies have demonstrated the role of the 5-HT_{2B} receptor in contributing to the therapeutic effects of selective serotonin reuptake inhibitors (SSRIs) antidepressants, and the 5-HT_{2B} receptor has been found to be necessary for the long-term behavioral effects of the antidepressant fluoxetine.²⁵

In addition, the 5-HT_{2B} receptor plays a protective role in the progression of amyotrophic lateral sclerosis²⁶ and Alzheimer's disease.²⁷

Therefore, PET studies of the 5-HT_{2B} receptor would be a powerful molecular tool to better understand its localization and role in these

different physiological and pathological processes.

To synthesize the first 5-HT_{2B} radiotracer, one of the challenges to be overcome is the lack of selectivity of many known 5-HT_{2B} ligands, due to the structural similarity between the 5-HT_{2B} receptor and other serotonin receptors, in particular 5-HT_{2A} and 5-HT_{2C}.

With a pK_i of 9.5 ± 0.1 for the human recombinant 5-HT_{2B} receptor, the antagonist RS-127445 has a 1000-fold selectivity over other receptors, making it one of the most selective 5-HT_{2B} receptor ligands known.^{28,29} RS-127445 has undergone clinical trials under the name of MT-500 for the prophylactic treatment of migraine and has shown antinociceptive activity in preclinical rodent studies.^{30,31} Although these trials did not lead to an approved treatment, RS-127445 is still the most widely used pharmacological tool to study the 5-HT_{2B} receptor and is found in the vast majority of scientific communications related to the 5-HT_{2B} receptor.

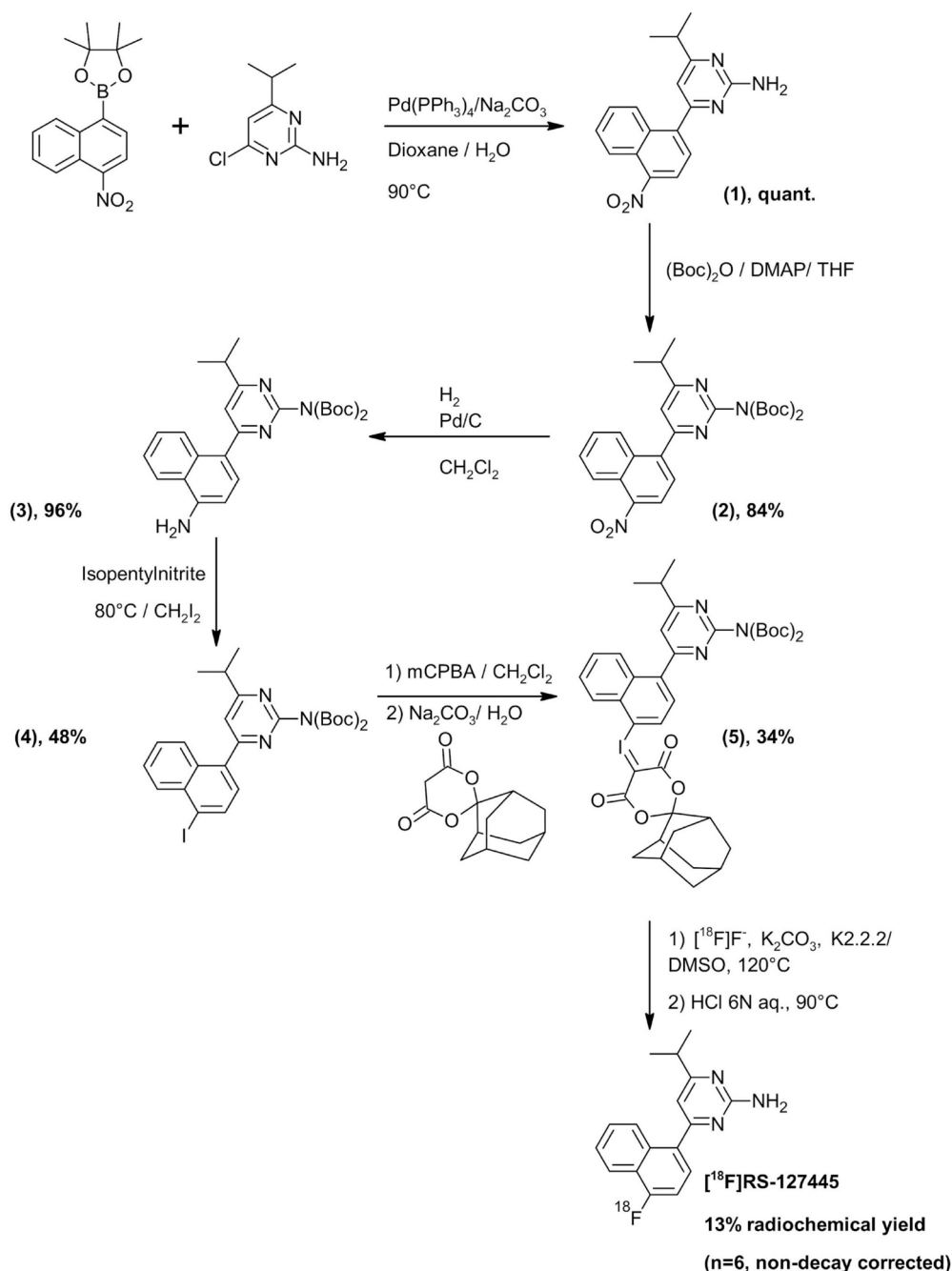


Fig. 1. Radiolabeling of RS-127445.

This molecule has been identified by other teams as having a great potential for PET ligand development,¹ and more recently, an analogue of RS-127445 has been described by replacing the amine group by a fluorine-18.³² However, this analogue has not been evaluated in PET imaging and is unlikely to have the same pharmacological properties as the parent compound, due to the loss of the important amine group. Therefore, in the present study we describe the first isotopic radiolabeling of RS-127445 and its preliminary evaluation as a 5-HT_{2B} radiotracer in rat brain.

We first attempted the radiolabeling of the nitro derivatives (1) and (2) (Fig. 1). Although the radiochemical purity and the molar activity of the radiotracer were sufficient, the radiochemical yield was low (<3%). We therefore decided to prepare the iodonium ylide precursor (5). The iodonium ylide group is known to have a better reactivity towards radiofluorination than the nitro group, especially when the latter is not activated by an electron withdrawing group at *ortho* or *para* position.³³ The radiolabeling of (5) by cyclotron produced [¹⁸F]fluoride was done at 120° C in DMSO in the presence of potassium carbonate and Kryptofix® 2.2.2, followed by deprotection of the amino group by aqueous hydrochloric acid. After purification by semipreparative HPLC and formulation in ethanol/water, [¹⁸F]RS-127445 was obtained in acceptable radiochemical yield (13.2 ± 1.8 % non-decay corrected, *n* = 6) and good radiochemical purity (>98 %) and molar activity (>100 GBq/μmol at the end of synthesis). The identity of the radiotracer was confirmed by semipreparative HPLC retention time, as well as analytical HPLC retention time. In addition, the radiotracer was found to be stable in ethanol/saline solution, for at least 3 h after the end of the synthesis.

The radiotracer was then evaluated in Sprague-Dawley rats using PET imaging. [¹⁸F]RS-127445 showed a high and rapid uptake in the rat brain. The SUV_{max} (standardized uptake value) was between 2.5 and 4, depending on the region. Peak uptake occurred within 5 min in all regions. However, the radiotracer showed a rapid clearance from the brain (Fig. 2).

The highest levels of SUVs were found in the brainstem, caudate putamen and the white matter of the cerebellum, while the lowest levels were observed in the various cortical regions (Fig. 3). This corresponds, in part, to what is known about 5-HT_{2B} receptor distribution, which has been identified in dorsal raphe nucleus (part of the brainstem)

but is thought to be low density in occipital cortex.¹⁸

To assess the specific binding of the radiotracer, we considered a homologous blocking with unlabeled RS-127445. This experiment requires solubilization of the molecule in a reasonable volume (<500 μL for rat experiments) of a suitable solvent prior to intravenous injection. RS-127445, in its commercial hydrochloride form, showed very low solubility in commonly used intravenous solvents, such as mixtures of DMSO (<10 % v/v) and isotonic aqueous solutions. We therefore decided to use the solvent described by Bonhaus *et al.*,²⁸ namely a mixture of propylene glycol/ethanol/water (5/1/4, v/v/v). Intravenous injections of 500 μL of this mixture in rats resulted in adverse effects in anesthetized rats, which precluded us from continuing the homologous blocking experiments by intravenous injection.

Consequently, intraperitoneal administration route was used for the homologous blocking experiment. This route allows for the injection of a greater solvent volume than intravenous route. In addition, the bioavailability of RS-127445 by intraperitoneal route has been already established.²⁸ RS-127445 was therefore solubilized in 0.3 % aqueous Tween® 80 and a dose of 1 mg/kg was administered to each rat 30 min before the radiotracer (total injected volume of competitor solution 2–3 mL). The results showed no blockade of the radiotracer binding (Supplementary Material).

Therefore, heterologous blocking experiments with LY-266097 were considered. This molecule is a selective antagonist of the 5-HT_{2B} receptor with a pKi which is comparable to that of RS-127445 (9.80) but with less selectivity towards other receptors (100-fold selectivity for 5-HT_{2B} vs 5-HT_{2A} and 5-HT_{2C}).³⁴ Fortunately, commercially available LY-266097 hydrochloride showed a satisfactory solubility in DMSO/isotonic aqueous glucose solution (5/95, v/v), which allowed intravenous injection.

Blocking experiments with intravenous pre-injection of 1 mg/kg LY-266097 resulted in a weak radiotracer shift (Figure 4).

Table 1 shows the blockade percentage in different regions, calculated on the mean SUVs from 30 to 60 min after radiotracer injection. This time interval was chosen to avoid the initial diffusion period. It should be noted that the use of other time intervals gave comparable results. No statistically significant blockade for any region was detected, but an overall trend of very low blockade was observed.

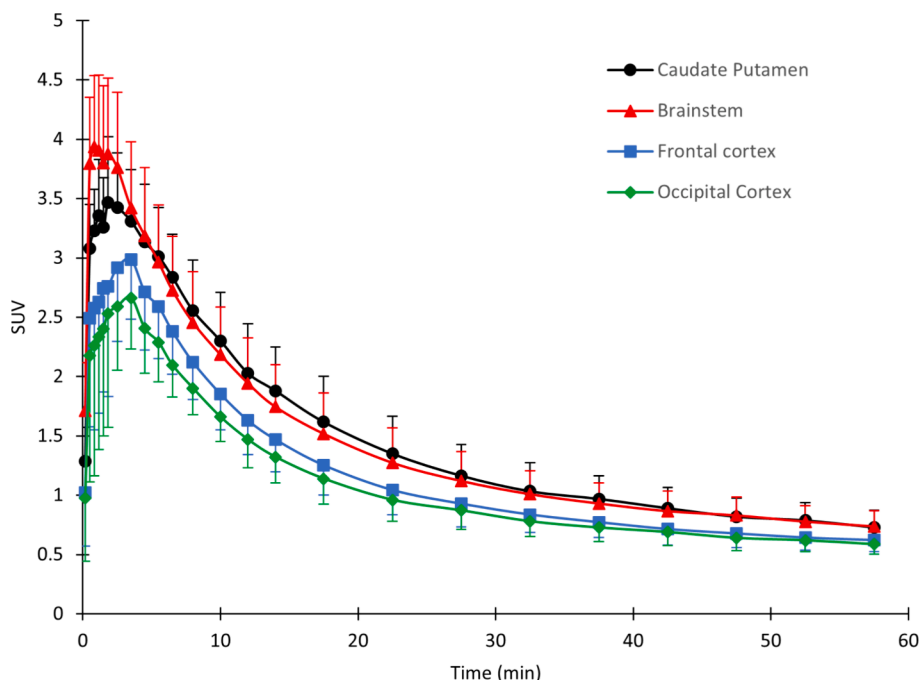


Fig. 2. Standardized uptake values (SUV) curves of [¹⁸F]RS-127445 in different rat brain regions, error bars represent the standard deviation (*n* = 8).

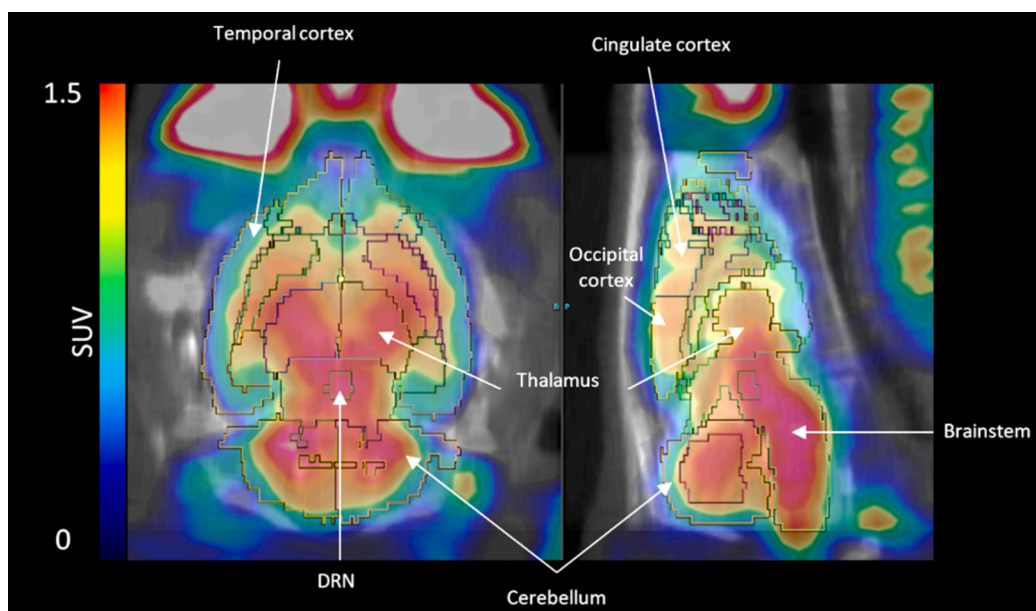


Fig. 3. Brain distribution of [¹⁸F]RS-127445 in a male Sprague-Dawley rat by PET imaging. Images are summed from 0 to 60 min (DRN: dorsal raphe nucleus).

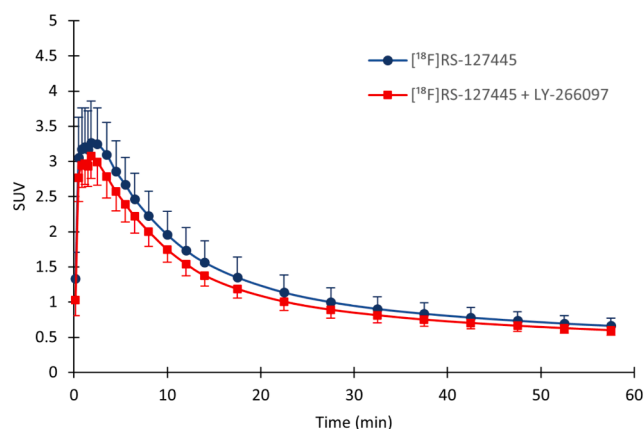


Fig. 4. Total rat brain activity curves of [¹⁸F]RS-127445 with radiotracer alone and after pretreatment with LY-266097 (1 mg/kg, iv) 2 min before radiotracer injection, error bars represent the standard deviation (n = 8).

To explore these results further, the SUV ratios (SUVr) were calculated using the occipital cortex as the reference region. This choice was based on the results showing the lowest SUV in the occipital cortex and the fact that the 5-HT_{2B} receptor is absent from the visual cortex¹⁸ (the region designated as the occipital cortex in the atlas used in our study includes the visual cortex). The results (Table 2) showed that the differences of SUVr are negligible in all brain regions.

In vitro autoradiography of rat brain and heart with increasing concentrations of RS-127445 (100 nM, 1 μM and 10 μM) in competition with [¹⁸F]RS-127445 showed no specific binding. Similar results were observed with LY-266097, PRX-08066 (another 5-HT_{2B} antagonist) and serotonin as competitors. Coronal and sagittal sections of different brain areas were tested (Fig. 5 and Supplementary material).

The low blocking by the competitors of brain labeling by the radiotracer is considered as an indicator of high ratio of the non-specific *versus* specific binding to the tissue. One of the possible reasons is independent of the radiotracer itself but depends on the target: a low density of the target receptor could explain the high non-specific fraction. Indeed, 5-HT_{2B} receptor is known to be less expressed in the brain than other

Table 1

Mean SUVs ± standard deviation from 30 to 60 min for baseline condition ([¹⁸F]RS-127445 alone) and after preinjection of LY-266097 (1 mg/kg, iv, 2 min before the radiotracer), n = 8. No significant difference was found between the two conditions for any region (Wilcoxon signed rank test, p = 0.05).

	Baseline	LY-266097	Blockade ^(a) by LY-266097
Caudate Putamen	0.87 ± 0.18	0.78 ± 0.10	(8 ± 22) %
Cerebellum White Matter	0.86 ± 0.14	0.78 ± 0.10	(7 ± 20) %
Brainstem	0.86 ± 0.16	0.77 ± 0.09	(7 ± 20) %
Thalamus	0.82 ± 0.14	0.75 ± 0.10	(6 ± 21) %
Hypothalamus	0.81 ± 0.15	0.73 ± 0.10	(8 ± 21) %
Cortex White Matter	0.77 ± 0.14	0.71 ± 0.10	(5 ± 21) %
Hippocampus	0.77 ± 0.13	0.69 ± 0.09	(7 ± 20) %
Whole Brain	0.77 ± 0.13	0.69 ± 0.09	(7 ± 20) %
Amygdala	0.77 ± 0.14	0.69 ± 0.09	(8 ± 21) %
Cingulate Cortex	0.76 ± 0.12	0.68 ± 0.09	(7 ± 21) %
Parietal Cortex	0.75 ± 0.14	0.66 ± 0.07	(9 ± 20) %
Cerebellar Cortex	0.73 ± 0.11	0.66 ± 0.08	(7 ± 19) %
Frontal Cortex	0.71 ± 0.12	0.62 ± 0.08	(10 ± 21) %
Temporal Cortex	0.68 ± 0.12	0.62 ± 0.07	(7 ± 20) %
Olfactory Bulb	0.68 ± 0.11	0.60 ± 0.08	(8 ± 19) %
Occipital Cortex	0.67 ± 0.11	0.63 ± 0.08	(5 ± 20) %

(a: Blockade percentages were calculated for each rat as (baseline SUV - pre-injection SUV)/baseline SUV, average blockade for 8 rats is shown ± standard deviation).

serotonin receptors, which explains the difficulty of detecting its presence in the rat brain in the oldest articles describing it.³⁵

Other hypotheses relate to the radiotracer itself. A high lipophilicity of the molecule could increase its non-specific binding to fatty regions in

Table 2

Mean SUVR ratios (SUVR) to the occipital cortex \pm standard deviation, calculated from 30 to 60 min for baseline condition ($[^{18}\text{F}]\text{RS-127445}$ alone) and after pre-injection of LY-266097 (1 mg/kg, iv) 5 min before the radiotracer (n = 8).

	Baseline	LY-266097	Difference ^(a)
Caudate Putamen	1.29 \pm 0.08	1.24 \pm 0.06	(4 \pm 5) %
Cerebellum White Matter	1.28 \pm 0.02	1.24 \pm 0.06	(3 \pm 4) %
Brainstem	1.27 \pm 0.05	1.24 \pm 0.07	(2 \pm 6) %
Thalamus Hypothalamus	1.22 \pm 0.05	1.20 \pm 0.07	(1 \pm 5) %
Cortex White Matter	1.21 \pm 0.05	1.16 \pm 0.05	(3 \pm 4) %
Hippocampus	1.14 \pm 0.05	1.14 \pm 0.05	(0 \pm 5) %
Whole Brain	1.14 \pm 0.04	1.11 \pm 0.04	(3 \pm 3) %
Amygdala	1.14 \pm 0.05	1.10 \pm 0.05	(4 \pm 6) %
Cingulate Cortex	1.13 \pm 0.08	1.09 \pm 0.04	(3 \pm 7) %
Parietal Cortex	1.10 \pm 0.05	1.05 \pm 0.03	(5 \pm 3) %
Cerebellar Cortex	1.09 \pm 0.02	1.06 \pm 0.05	(3 \pm 4) %
Frontal Cortex	1.05 \pm 0.06	0.98 \pm 0.02	(6 \pm 6) %
Temporal Cortex	1.01 \pm 0.04	0.99 \pm 0.04	(2 \pm 4) %
Olfactory Bulb	1.00 \pm 0.04	0.97 \pm 0.04	(4 \pm 4) %

a: Differences were calculated for each rat as (baseline SUVR - preinjection SUVR)/baseline SUVR, the average difference for 8 rats is shown \pm standard deviation.

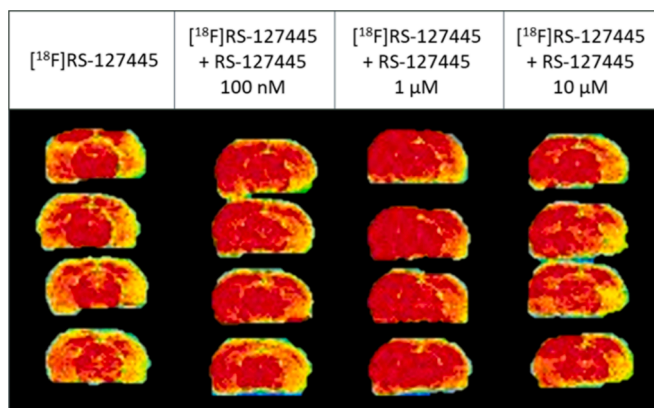


Fig. 5. Autoradiography of coronal brain sections of Sprague-Dawley rats. $[^{18}\text{F}]\text{RS-127445}$ was incubated with increasing concentrations of RS-127445. Sections are presented in quadruplicate.

the brain, thereby reducing the displaceable signal. Similarly, a high metabolism of the radiotracer and the presence of cerebral radiometabolites could be another explanation for the increase of the non-specific signal in the *in vivo* PET imaging studies.

In order to assess the lipophilicity of the molecule, and given that there is no literature describing an experimental calculation of RS-127445 logP, a shake-flask method was used to measure it, and a logP of 4.16 was found. This result is in agreement with the software prediction of 4.19, indicating that RS-127445 is a highly lipophilic molecule. Although there are no known limits to the lipophilicity of brain radiotracers, the measured logP value for RS-127445 is within the high lipophilicity values of known radiotracers, and could explain a high non-specific binding.³⁶ This high lipophilicity of the radiotracer could also explain the high level of binding to white matter regions of the brain observed during our PET experiments (Table 1).

In addition, the metabolism of the radiotracer was evaluated, and the results are summarized in Table 3. Thirty minutes after intravenous injection, less than the half of the radioactivity within the brain was due to the unmetabolized radiotracer. These findings are consistent with unmetabolized radiotracer in rat plasma after one hour, indicating a rapid metabolism of the molecule.

Experimental conditions could also influence the results of *in vivo* evaluation of the radiotracer. In fact, the anesthetic used in our study (isoflurane) was shown to interact with 5-HT_{2B} receptor, although the

Table 3

Percentage of unchanged radiotracer in rat brain at 5 and 30 min after $[^{18}\text{F}]\text{RS-127445}$ injection and in plasma, 60 min after $[^{18}\text{F}]\text{RS-127445}$ injection.

Brain		Plasma
5 min (n = 3)	30 min (n = 4)	60 min (n = 4)
86.3 \pm 4.9	45.8 \pm 5.6	35.5 \pm 3.7

affinity is low ($K_i = 7.78 \mu\text{M}$).³⁷ Isoflurane has already been used in several studies implicating 5-HT_{2B},^{18,23,38,39} and RS-127445 antagonist activity was not altered. Nevertheless, the hypothesis that isoflurane could have influenced the binding of $[^{18}\text{F}]\text{RS-127445}$ could not be completely ignored.

Finally, it should be noted that the affinity and specificity of RS-127445 toward rat 5-HT_{2B} receptor is, at our knowledge, not known, unlike its affinity toward the human analog which is well documented.^{28,29} Available literature indicates a high antagonist effect toward rat 5-HT_{2B} receptor assessed by the inhibition of rat stomach fundus contraction and rat jugular vein relaxation, with pA₂ values of 9.5 \pm 1.1 and 9.95 \pm 0.07 respectively.²⁸ However, the hypothesis that the low specific binding of $[^{18}\text{F}]\text{RS-127445}$ could result from a low affinity or a low specificity toward rat 5-HT_{2B} receptor, while it is not our privileged hypothesis, should not be totally excluded.

In conclusion, in this work we describe the first radiosynthesis of $[^{18}\text{F}]\text{RS-127445}$ and its preliminary evaluation as a 5-HT_{2B} radiotracer in rat brain. While PET images showed a good brain penetration and a labeling that roughly corresponded to the known distribution of 5-HT_{2B} receptor (with high labeling in brainstem regions compared to cortical regions), the displacement with a competitor antagonist was very low in all regions. This could be due either to the high non-specific binding of the radiotracer or its metabolites, or to the low density of the 5-HT_{2B} receptor. These results suggest that $[^{18}\text{F}]\text{RS-127445}$ may be an inappropriate radiotracer in rat brain. However, this does not preclude its evaluation in other species, as metabolism and non-specific binding are known to be species-dependent properties.⁴⁰

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

Data will be made available on request.

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Appendix A. Supplementary data

Experimental details, additional autoradiographies, chromatograms of radiotracer purification and control, as well as NMR spectra of all newly synthesized compounds are provided. Supplementary data to this article can be found online at <https://doi.org/10.1016/j.bmcl.2024.129933>.

References

1. Fu H, Rong J, Chen Z, Zhou J, Collier T, Liang SH. Positron Emission Tomography (PET) Imaging Tracers for Serotonin Receptors. *J Med Chem*. 2022;65:10755–10808. <https://doi.org/10.1021/acs.jmedchem.2c00633>.
2. Lancelot S, Zimmer L. Small-animal positron emission tomography as a tool for neuropharmacology. *Trends Pharmacol Sci*. 2010;31:411–417. <https://doi.org/10.1016/j.tips.2010.06.002>.
3. Mangeant R, Dubost E, Cailly T, Collot V. Radiotracers for the Central Serotonergic System. *Pharmaceuticals*. 2022;15:571. <https://doi.org/10.3390/ph15050571>.
4. Kursar D, Nelson L, Waincott B, Cohen L. Molecular Cloning, Functional Expression, and Pharmacological Characterization of a Novel Serotonin Receptor (5-Hydroxytryptamine_{2F}) from Rat Stomach Fundus. *Mol Pharmacol*. 1992;42:549–557.
5. Devroye C, Cathala A, Piazza PV, Spampinato U. The central serotonin_{2B} receptor as a new pharmacological target for the treatment of dopamine-related neuropsychiatric disorders: Rationale and current status of research. *Pharmacol Ther*. 2018;181:143–155. <https://doi.org/10.1016/j.pharmthera.2017.07.014>.
6. Bonaventure P, Nepomuceno D, Miller K, et al. Molecular and pharmacological characterization of serotonin 5-HT_{2A} and 5-HT_{2B} receptor subtypes in dog. *Eur J Pharmacol*. 2005;513:181–192. <https://doi.org/10.1016/j.ejphar.2005.03.013>.
7. Etienne N, Schaeerlinger B, Jaffré F, Maroteaux L. The 5-HT_{2B} receptor: a main cardio-pulmonary target of serotonin. *J Soc Biol*. 2004;198:22–29.
8. Schmuck K, Ullmer C, Engels P, Lübbert H. Cloning and functional characterization of the human 5-HT_{2B} serotonin receptor. *FEBS Lett*. 1994;342:85–90. [https://doi.org/10.1016/0014-5793\(94\)80590-3](https://doi.org/10.1016/0014-5793(94)80590-3).
9. Bonhaus DW, Bach C, DeSouza A, et al. The pharmacology and distribution of human 5-hydroxytryptamine_{2B} (5-HT_{2B}) receptor gene products: comparison with 5-HT_{2A} and 5-HT_{2C} receptors. *Br J Pharmacol*. 1995;115:622–628. <https://doi.org/10.1111/j.1476-5381.1995.tb14977.x>.
10. Bonaventure P, Guo H, Tian B, et al. Nuclei and subnuclei gene expression profiling in mammalian brain. *Brain Res*. 2002;943:38–47. [https://doi.org/10.1016/S0006-8993\(02\)02504-0](https://doi.org/10.1016/S0006-8993(02)02504-0).
11. Ullmer C, Schmuck K, Kalkman HO, Lübbert H. Expression of serotonin receptor mRNAs in blood vessels. *FEBS Lett*. 1995;370:215–221. [https://doi.org/10.1016/0014-5793\(95\)00828-W](https://doi.org/10.1016/0014-5793(95)00828-W).
12. Berger M, Gray JA, Roth BL. The Expanded Biology of Serotonin. *Annu Rev Med*. 2009;60:355–366. <https://doi.org/10.1146/annurev.med.60.042307.110802>.
13. Rothman RB, Baumann MH, Savage JE, et al. Evidence for Possible Involvement of 5-HT_{2B} Receptors in the Cardiac Valvulopathy Associated With Fenfluramine and Other Serotonergic Medications. *Circulation*. 2000;102:2836–2841. <https://doi.org/10.1161/01.CIR.102.23.2836>.
14. Szymanski C, Andréjak M, Peltier M, Maréchaux S, Tribouilloy C. Adverse effects of benfluorex on heart valves and pulmonary circulation. *Pharmacoeconom Drug Saf*. 2014;23:679–686. <https://doi.org/10.1002/pds.3642>.
15. Andrejak M, Tribouilloy C. Drug-induced valvular heart disease: An update. *Archiv Cardiovasc Dis*. 2013;106:333–339. <https://doi.org/10.1016/j.acvd.2013.02.003>.
16. Rouaud A, Calder AE, Hasler G. Microdosing psychedelics and the risk of cardiac fibrosis and valvulopathy: Comparison to known cardiotoxins. *J Psychopharmacol Oxf Engl*. 2024;38:217–224. <https://doi.org/10.1177/02698811231225609>.
17. Auclair AL, Cathala A, Sarrazin F, et al. The central serotonin_{2B} receptor: a new pharmacological target to modulate the mesoaccumbens dopaminergic pathway activity. *J Neurochem*. 2010;114:1323–1332. <https://doi.org/10.1111/j.1471-4159.2010.06848.x>.
18. Cathala A, Lucas G, López-Terrones E, Revest JM, Artigas F, Spampinato U. Differential expression of serotonin_{2B} receptors in GABAergic and serotonergic neurons of the rat and mouse dorsal raphe nucleus. *Mol Cell Neurosci*. 2022;121, 103750. <https://doi.org/10.1016/j.mcn.2022.103750>.
19. Duxon MS, Flanigan TP, Reavley AC, Baxter GS, Blackburn TP, Fone KCF. Evidence for expression of the 5-hydroxytryptamine-2B receptor protein in the rat central nervous system. *Neuroscience*. 1997;76:323–329. [https://doi.org/10.1016/S0306-4522\(96\)00480-0](https://doi.org/10.1016/S0306-4522(96)00480-0).
20. Li B, Dong L, Wang B, Cai L, Jiang N, Peng L. Cell Type-Specific Gene Expression and Editing Responses to Chronic Fluoxetine Treatment in the In Vivo Mouse Brain and Their Relevance for Stress-Induced Anhedonia. *Neurochem Res*. 2012;37:2480–2495. <https://doi.org/10.1007/s11064-012-0814-1>.
21. Kolodziejczak M, Béchade C, Gervasi N, et al. Serotonin Modulates Developmental Microglia via 5-HT_{2B} Receptors: Potential Implication during Synaptic Refinement of Retinogeniculate Projections. *ACS Chem Neurosci*. 2015;6:1219–1230. <https://doi.org/10.1021/cn5003489>.
22. Devroye C, Cathala A, Haddjeri N, et al. Differential control of dopamine ascending pathways by serotonin_{2B} receptor antagonists: New opportunities for the treatment of schizophrenia. *Neuropharmacology*. 2016;109:59–68. <https://doi.org/10.1016/j.neuropharm.2016.05.024>.
23. Cathala A, Devroye C, Robert É, et al. Serotonin_{2B} receptor blockade in the rat dorsal raphe nucleus suppresses cocaine-induced hyperlocomotion through an opposite control of mesocortical and mesoaccumbens dopamine pathways. *Neuropharmacology*. 2020;180, 108309. <https://doi.org/10.1016/j.neuropharm.2020.108309>.
24. Doly S, Valjent E, Setola V, et al. Serotonin 5-HT_{2B} Receptors Are Required for 3,4-Methylenedioxymethamphetamine-Induced Hyperlocomotion and 5-HT Release In Vivo and In Vitro. *J Neurosci*. 2008;28:2933–2940. <https://doi.org/10.1523/JNEUROSCI.5723-07.2008>.
25. Hertz L, Rothman DL, Li B, Peng L. Chronic SSRI stimulation of astrocytic 5-HT_{2B} receptors change multiple gene expressions/editings and metabolism of glutamate, glucose and glycogen: a potential paradigm shift. *Front Behav Neurosci*. 2015;9. <https://doi.org/10.3389/fnbeh.2015.00025>.
26. El Oussini H, Bayer H, Scekic-Zahirovic J, et al. Serotonin 2B receptor slows disease progression and prevents degeneration of spinal cord mononuclear phagocytes in amyotrophic lateral sclerosis. *Acta Neuropathol (Berl)*. 2016;131:465–480. <https://doi.org/10.1007/s00401-016-1534-4>.
27. Maroteaux L, Monassier L, eds. *5-HT_{2B} Receptors: from Molecular Biology to Clinical Applications*. Springer International Publishing; 2021. <https://doi.org/10.1007/978-3-030-55920-5>.
28. Bonhaus DW, Flippin LA, Greenhouse RJ, et al. RS-127445: a selective, high affinity, orally bioavailable 5-HT_{2B} receptor antagonist. *Br J Pharmacol*. 1999;127:1075–1082. <https://doi.org/10.1038/sj.bjp.0702632>.
29. Antony R K, Misra A, Quirk K, et al. Pharmacological characterisation of the agonist radioligand binding site of 5-HT_{2A}, 5-HT_{2B} and 5-HT_{2C} receptors. *Naunyn Schmiedebergs Arch Pharmacol*. 2004;370:114–123. <https://doi.org/10.1007/s00210-004-0951-4>.
30. HA Mucke. MT-500 (POZEN). *Curr Opin Investig Drugs Lond Engl*. 2001;2(3):419–421.
31. Cervantes-Durán C, Pineda-Farías JB, Bravo-Hernández M, et al. Evidence for the participation of peripheral 5-HT_{2A}, 5-HT_{2B}, and 5-HT_{2C} receptors in formalin-induced secondary mechanical allodynia and hyperalgesia. *Neuroscience*. 2013;232:169–181. <https://doi.org/10.1016/j.neuroscience.2012.11.047>.
32. Ford J, Ortall S, Chen Z, Sap J, Tredwell M, Gouverneur V. Expedient Access to 18F-Fluoroheteroarenes via Deaminative Radiofluorination of Aniline-Derived Pyridinium Salts. *Angew Chem Int Ed. Published Online*. 2024:e202404945.
33. Rotstein BH, Wang L, Liu RY, et al. Mechanistic studies and radiofluorination of structurally diverse pharmaceuticals with spirocyclic iodonium(III)ylides. *Chem Sci*. 2016;7:4407–4417. <https://doi.org/10.1039/C6SC00197A>.
34. Audia JE, Evrard DA, Murdoch GR, et al. Potent, Selective Tetrahydro-β-carboline Antagonists of the Serotonin 2B (5HT_{2B}) Contractile Receptor in the Rat Stomach Fundus. *J Med Chem*. 1996;39:2773–2780. <https://doi.org/10.1021/jm960062t>.
35. Pompeiano M, Palacios JM, Mengod G. Distribution of the serotonin 5-HT₂ receptor family mRNAs: comparison between 5-HT_{2A} and 5-HT_{2C} receptors. *Mol Brain Res*. 1994;23:163–178. [https://doi.org/10.1016/0169-328X\(94\)90223-2](https://doi.org/10.1016/0169-328X(94)90223-2).
36. Pike VW. PET radiotracers: crossing the blood–brain barrier and surviving metabolism. *Trends Pharmacol Sci*. 2009;30:431–440. <https://doi.org/10.1016/j.tips.2009.05.005>.
37. Matsunaga F, Gao L, Huang XP, Saven JG, Roth BL, Liu R. Molecular interactions between general anesthetics and the 5HT_{2B} receptor. *J Biomol Struct Dyn*. 2015;33:211–218. <https://doi.org/10.1080/07391102.2013.869483>.
38. Urtikova N, Berson N, Van Steenwinckel J, et al. Antinociceptive effect of peripheral serotonin 5-HT_{2B} receptor activation on neuropathic pain. *Pain*. 2012;153:1320–1331. <https://doi.org/10.1016/j.pain.2012.03.024>.
39. Devroye C, Haddjeri N, Cathala A, et al. Opposite control of mesocortical and mesoaccumbal dopamine pathways by serotonin 2B receptor blockade: Involvement of medial prefrontal cortex serotonin 1A receptors. *Neuropharmacology*. 2017;119:91–99. <https://doi.org/10.1016/j.neuropharm.2017.04.001>.
40. Van De Bittner GC, Ricq EL, Hooker JM. A Philosophy for CNS Radiotracer Design. *Acc Chem Res*. 2014;47:3127–3134. <https://doi.org/10.1021/ar500233s>.