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Florian Ferreri, Dominique Drapier, Emmanuelle Baloche, Mehemed Ouzid, Luc Zimmer, et al.. The in Vitro Actions of Loxapine on Dopaminergic and Serotonergic Receptors. Time to Consider Atypical Classification of This Antipsychotic Drug?. *International Journal of Neuropsychopharmacology*, 2017, 21 (4), pp.355-360. 10.1093/ijnp/pyx102 . hal-01696767

**HAL Id: hal-01696767**

**<https://uca.hal.science/hal-01696767v1>**

Submitted on 21 Jun 2018

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## BRIEF REPORT

# The in Vitro Actions of Loxapine on Dopaminergic and Serotonergic Receptors. Time to Consider Atypical Classification of This Antipsychotic Drug?

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## Abstract

**Background:** The denomination of typical antipsychotic for loxapine has poor relation to current knowledge of the molecule's relevant modes of action.

**Materials and Methods:** Competition binding experiments were performed on expressed human recombinant receptors in CHO cells and HEK-293 cells for D<sub>1</sub> to D<sub>5</sub>, 5-HT<sub>1A</sub>, 5-HT<sub>2A</sub>, 5-HT<sub>2C</sub>, 5-HT<sub>4</sub>, 5-HT<sub>6</sub>, and 5-HT<sub>7</sub>. In vitro autoradiographies using [<sup>11</sup>C]-Raclopride [<sup>18</sup>F]-Altanserin [<sup>18</sup>F]-MPPF [<sup>11</sup>C]-SB207145, and [<sup>18</sup>F]-2FNQ1P were measured in brain tissue of a male primate followed by addition of increasing doses of loxapine succinate.

**Results:** In cell cultures, the measured K<sub>b</sub> confirmed high affinity of loxapine for the D<sub>2</sub>; intermediate affinity for the D<sub>1</sub>, D<sub>4</sub>, D<sub>5</sub>, 5-HT<sub>2C</sub> receptors and a lack of affinity toward D<sub>3</sub>, 5-HT<sub>1A</sub>, 5-HT<sub>4</sub>, 5-HT<sub>6</sub>, and 5-HT<sub>7</sub> receptors. In brain tissue, PET autoradiographies showed a radiopharmaceutical displacement at low concentrations of loxapine on D<sub>2</sub> and 5-HT<sub>2A</sub> receptors. **Conclusion:** This preclinical study reveals that loxapine receptorial spectrum is close to an “atypical” profile (D<sub>2</sub>/5HT<sub>2A</sub> ratio, 1.14). Loxapine is rightly classified as a DS-RAN agent in the Neuroscience Based Nomenclature classification.

**Keywords:** loxapine, binding, PET radiopharmaceutical, dopamine receptor, serotonin receptor

## Introduction

Loxapine is considered as a well-known first-generation antipsychotic. In terms of chemistry, loxapine is a dibenzoxazepine molecule, with a tricyclic structure chemically close to clozapine. This molecule, initially released in the mid-1970s, is available for oral, i.m., and, more recently, oral inhalation use (Keating, 2013).

Loxapine has been used for the treatment of schizophrenia and agitated adult since the 1970s. The recent approval by regulatory agencies in the United States and European Union of loxapine inhalation powder in the acute treatment of agitated adult patients with schizophrenia or bipolar disorder renews interest in this molecule (Keating, 2013; Popovic et al., 2015).

Received: June 6, 2017; Revised: October 15, 2017; Accepted: October 29, 2017

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## Significance Statement

This article is an update of the dopamine-serotonin receptor binding characteristics of loxapine. At low doses, loxapine is shown to be in vitro close to an atypical profile, since its 5-HT<sub>2A</sub> binding occupancy is higher than its D<sub>2</sub> binding (high 5-HT<sub>2A</sub>/D<sub>2</sub> ratio). These in vitro results are confirmed in nonhuman primates with PET radiopharmaceuticals. The in vivo PET study revealed that loxapine has possibly a significant affinity for other serotonergic receptors (5-HT<sub>1A</sub> and 5-HT<sub>6</sub>).

Currently, prescribers interested in this drug have little pharmacological information at their disposal (Popovic et al., 2015). A systematic literature search about the pharmacological properties of loxapine leads to a small number of publications, mainly with old data not recently updated and hard to reach online. Particularly, the receptor binding spectrum of loxapine is only documented by public data available in the PDSD Ki Database (National Institute of Mental Health).

Among these sparse data, it appears that loxapine is usually classified as a “typical” antipsychotic (or first-generation antipsychotic). Its antipsychotic efficacy is proposed to be mainly mediated through antagonism of postsynaptic dopamine D<sub>2</sub> receptors. This status, shared by the majority of first-generation neuroleptics, explains that loxapine is essentially prescribed as a second or third choice antipsychotic.

Several authors have proposed that loxapine has “atypical” characteristics (Glazer, 1999; Chakrabarti et al., 2007). The atypicality of an antipsychotic is mainly but not only defined by its receptor binding profile, especially the high-affinity antagonism of serotonin 5-HT<sub>2A</sub> in complement to dopamine D<sub>2</sub> receptor antagonism, leading to a high 5-HT<sub>2A</sub>/D<sub>2</sub> occupancy ratio (Meltzer et al., 1989). However, no recent experimental data have been released to confirm this new classification of loxapine.

As a consequence, the in vitro receptor binding profile of typical and atypical antipsychotic drugs is frequently without any mention to loxapine (Kusumi et al., 2015). This poor literature is a real drawback for a comprehensive and evidence-based medicine loxapine prescription, pharmaceutical processing, and monitoring of utilization.

Neuropharmacology is a major tool to manage side effects, switches, and molecule association (e.g., introduction or cessation of the loxapine administration). The knowledge of the neuropharmacological profile of loxapine makes us all the more interested in the question, since the classical classification is currently in evolution. Some of the major international neuropsychopharmacology associations (ECNP, ACNP, Asian CNP, and CINP) propose a modification of psychotropic classification. Its aim is to address a longstanding concern within the neuropsychopharmacological community and psychiatric communities that the nomenclature of current psychotropic drugs, including antipsychotics, does not properly reflect the underlying mechanism of action of these compounds (Zohar et al., 2015). The Neuroscience Based Nomenclature proposes a new template placing pharmacology rather than indication as the primary axe.

In this emerging and challenging context, the purpose of our study is to describe loxapine's serotonin and dopamine receptor occupancy in vitro using various techniques. The first aim was to focus on dopamine and serotonin receptors, crucial in the antipsychotic effect, in a classical receptor binding strategy. The second aim was to transfer this receptor exploration toward in vitro positron emission tomography neuroimaging (PET) by using clinical radiopharmaceutical in autoradiography performed with nonhuman primate brain tissues.

## Materials and Methods

### Receptor Binding Protocols

In a first step, the respective affinities (K<sub>b</sub>) of loxapine succinate (provided by EISAI) were determined toward dopamine receptors (D<sub>1</sub>, D<sub>2</sub>, D<sub>3</sub>, D<sub>4</sub>, and D<sub>5</sub>) and serotonin receptors (5-HT<sub>1A</sub>, 5-HT<sub>2A</sub>, 5-HT<sub>2C</sub>, 5-HT<sub>4</sub>, 5-HT<sub>6</sub>, and 5-HT<sub>7</sub>) by Cerep, an in vitro pharmacology company (<http://www.cerep.fr>). Competition binding experiments were performed on expressed human recombinant receptors in CHO and HEK-293 cells (vs SCH 23390, butaclamol, (+)butaclamol, clozapine, SCH2390, WAY100635, ketanserin, SB206553, GR113808, methiothepin, for D<sub>1</sub>, D<sub>2</sub>, D<sub>3</sub>, D<sub>4</sub>, D<sub>5</sub>, 5-HT<sub>1A</sub>, 5-HT<sub>2A</sub>, 5-HT<sub>2C</sub>, 5-HT<sub>4</sub>, 5-HT<sub>6</sub>, and 5-HT<sub>7</sub> receptors, respectively). Each experiment was performed in duplicate at 5 different concentrations. IC<sub>50</sub> values (concentration eliciting half-maximal inhibition of the control specific agonist response) were determined by nonlinear regression analysis of the concentration-response curves generated with mean replicate values using Hill equation curve fitting. Analyses used the commercial SigmaPlot 11.0 software (Systat Software Inc.). The K<sub>b</sub> values were calculated using the modified Cheng Prusoff equation (see <https://www.eurofins.com/biopharma-services/> for supplementary experimental details).

### PET Radiopharmaceutical Syntheses and Quality Controls

On the days of autoradiography experiments, PET radiopharmaceuticals were produced and handled in the CERMEP-Imaging Platform. [<sup>11</sup>C]-Raclopride and [<sup>11</sup>C]-SB207145 radiopharmaceuticals were obtained after [<sup>11</sup>C]-methylation of their respective chemical precursors in an automated synthesizer for <sup>11</sup>C-PET radiopharmaceuticals (Scansys) according to methods previously published (Hassoun et al., 2003; Madsen et al., 2011). [<sup>18</sup>F]-altanserin (5-HT<sub>2A</sub> receptors), [<sup>18</sup>F]-MPPF (5-HT<sub>1A</sub> receptors), and [<sup>18</sup>F]-2FNQ1P (5-HT<sub>6</sub> receptors) radiopharmaceuticals were radiolabeled by [<sup>18</sup>F]-nucleophilic substitution of their respective chemical precursors in an automated synthesizer for <sup>18</sup>F-fluorinated PET radiopharmaceuticals (Ora) according to previously described radiochemical pathways (Haugbol et al., 2007; Becker et al., 2014, 2015). Their chemical and radiochemical purities, as measured by HPLC, were >98%. The specific activity at time of autoradiography was >37 GBq/mmol (1 Ci/mmol) for all PET radiopharmaceuticals.

### Autoradiography Protocols and Image Analyses

A nonhuman male primate (cynomolgus monkey; 6 kg body weight) was used for this study in accordance with European guidelines for care of laboratory animals (2010/63/EU) and with the ethics committee of the University of Lyon (Université Claude Bernard Lyon 1). After killing by an overdose isoflurane inhalation, the primate brain was carefully removed and immediately frozen in 2-methylbutane cooled with dry ice (−29°C). Briefly, horizontal sections (30 μm thick)

cut across the hippocampus and the striatal region were carried out using a  $-20^{\circ}\text{C}$ -cryostat (Microm-Microtech), thaw-mounted on glass slides, and allowed to air dry before storage at  $-80^{\circ}\text{C}$  until used.

On the days of radiosynthesis, the slides were allowed to reach ambient temperature and then incubated for 20 minutes in Tris-phosphate-buffered saline buffer (138 mM NaCl, 2.7 mM KCl, pH adjusted to 7.6) containing 37 kBq/mL (1  $\mu\text{Ci}/\text{mL}$ ) of the radiopharmaceutical, e.g., [ $^{11}\text{C}$ ]-Raclopride, [ $^{18}\text{F}$ ]-Altanserin, [ $^{18}\text{F}$ ]-MPPF, [ $^{11}\text{C}$ ]-SB207145, and [ $^{18}\text{F}$ ]-2FNQ1P.

For competition experiments, the slides were placed in the same buffer supplemented with loxapine succinate (1 nM, 10 nM, 50 nM, 100 nM, and 1  $\mu\text{M}$ ). After incubation, the slides were dipped in cold buffer ( $4^{\circ}\text{C}$ ) for 90 seconds, in distilled cold water ( $4^{\circ}\text{C}$ ) for 90 seconds, then dried and juxtaposed to a phosphor imaging plate for 60 minutes (BAS-1800 II, Fujifilm). Regions of interest (cortical regions, caudate, putamen, and hippocampus) were drawn manually using Multigauge software (Fujifilm). The results were expressed in arbitrary units.

## Results

### Receptor Binding Protocols

The measured  $K_b$  demonstrated high affinity of loxapine succinate for the  $D_2$  receptor and  $5\text{-HT}_{2A}$  receptor with a value  $<2$  nM (Figure 1). Loxapine succinate presented intermediate affinity for the  $D_1$ ,  $D_4$ ,  $D_5$ , and  $5\text{-HT}_{2C}$  receptors, with a  $K_b$  value between 12 and 29 nM. In these experimental conditions, loxapine succinate presented total lack of affinity toward  $D_3$ ,  $5\text{-HT}_{1A}$ ,  $5\text{-HT}_4$ ,  $5\text{-HT}_6$ , and  $5\text{-HT}_7$  receptors ( $K_b > 1$   $\mu\text{M}$ ).

### PET Autoradiography

The autoradiography distribution of receptor radiotracers varied across the striatal regions for [ $^{11}\text{C}$ ]-Raclopride, [ $^{11}\text{C}$ ]-SB207145, and [ $^{18}\text{F}$ ]-2FNQ1P, according to the known distribution of  $D_2$ ,  $5\text{-HT}_4$ , and  $5\text{-HT}_6$  receptors, respectively. The distribution of [ $^{18}\text{F}$ ]-Altanserin was cortical as known for  $5\text{-HT}_{2A}$  receptor location. Finally, the binding of [ $^{18}\text{F}$ ]-MPPF was correlated with the known distribution of  $5\text{-HT}_{1A}$  receptors, mainly located in hippocampus (Figure 2).

These PET autoradiographies allow the measurement of radiopharmaceutical displacements/competition at different concentrations of loxapine succinate. A competition occurred at low concentrations of loxapine on  $D_2$  receptors ( $-50\%$  of [ $^{11}\text{C}$ ]-Raclopride displacement at 10 nM,  $-80\%$  at 50 nM, and  $-90\%$  at 100 nM of loxapine, respectively). In a similar manner, the displacement of [ $^{18}\text{F}$ ]-altanserin ( $5\text{-HT}_{2A}$  receptors) was significant at 10, 50, and 100 nM of loxapine ( $-30\%$ ). Radiopharmaceutical displacements were observed at higher concentrations of loxapine on  $5\text{-HT}_{1A}$  receptors ( $-40\%$  at 100 nM and  $-90\%$  of [ $^{18}\text{F}$ ]-MPPF at 1  $\mu\text{M}$  of loxapine) and  $5\text{-HT}_6$  receptors ( $-20\%$  of [ $^{18}\text{F}$ ]-2FNQ1P at 50 and 100 nM of loxapine, respectively). No effect was measured on  $5\text{-HT}_4$  receptors ([ $^{11}\text{C}$ ]-SB207145).

### Discussion

The aim of the first part of this study was to have an updated overview of the receptor binding spectrum of loxapine focused on the classical receptors involved in the antipsychotic effects (e.g.,  $D_2$ ,  $5\text{-HT}_{2A}$  receptors...) on other receptors involved in complementary psychopharmacological effects (e.g.,  $5\text{-HT}_4$ ,  $5\text{-HT}_6$ ,  $5\text{-HT}_7$  receptors...) or receptors responsible of possible

Dopamine Receptors	Loxapine Affinity (Kb)
$D_1$	29 nM
$D_2$	2.4 nM
$D_3$	NS
$D_4$	12 nM
$D_5$	28 nM

Serotonin Receptors	Loxapine Affinity (Kb)
$5\text{-HT}_{1A}$	NS
$5\text{-HT}_{2A}$	2.1 nM
$5\text{-HT}_{2C}$	22 nM
$5\text{-HT}_4$	NS
$5\text{-HT}_6$	NS
$5\text{-HT}_7$	NS

Figure 1: Profiles of loxapine affinity toward dopaminergic and serotonergic receptors.  $K_b$  values were generated from competition studies performed on human recombinant receptors expressed in CHO or HEK-293 cells. (NS, non-significant).

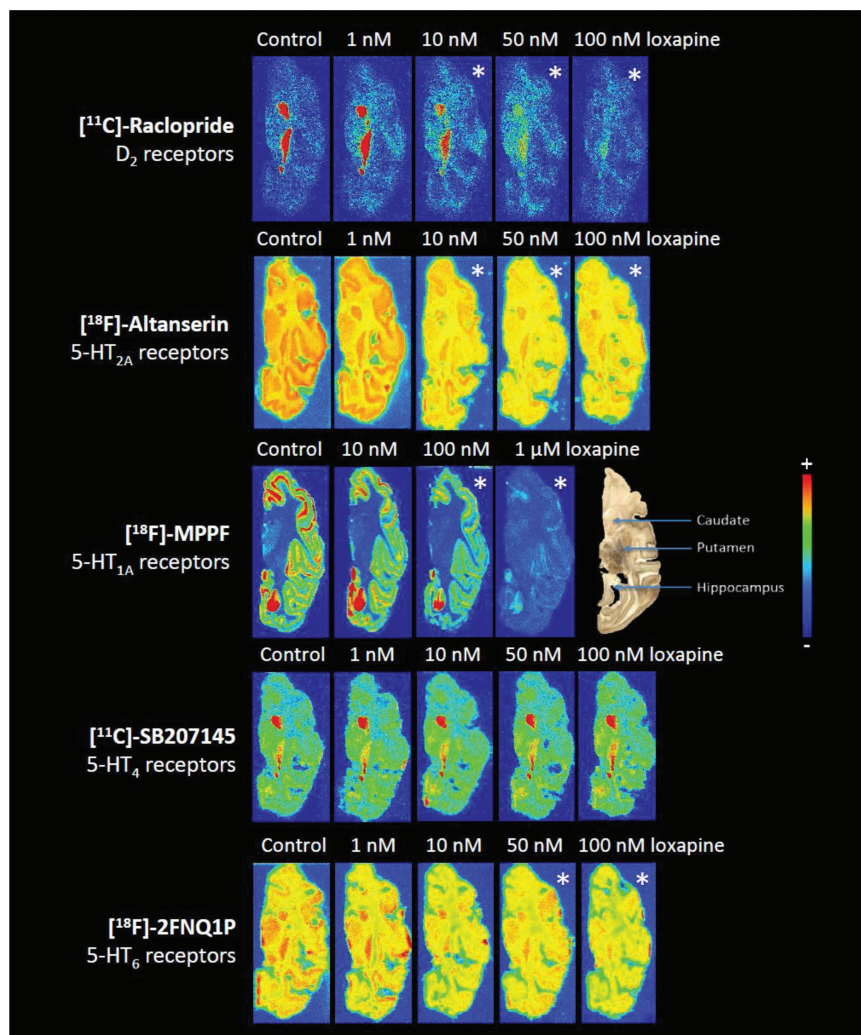


Figure 2: Dopaminergic and serotonergic PET radiopharmaceutical in vitro distributions in macaque brain. Monkey cerebral sections were processed for in vitro autoradiography with [ $^{11}\text{C}$ ]-Raclopride, [ $^{18}\text{F}$ ]-Altanserin, [ $^{18}\text{F}$ ]-MPPF, [ $^{11}\text{C}$ ]-SB207145 and [ $^{18}\text{F}$ ]-2FNQ1P under baseline condition and in presence of increasing concentrations of loxapine. \* indicate a significant decrease of the radiopharmaceutical binding ( $p < 0.05$ ).

side effects ( $H_1$ ,  $M_1$  receptors...). A total of 20 targets have been explored in vitro in cellular cultures. The aim of the second part of this study was more translational with the use of PET radiopharmaceuticals in nonhuman brain tissues focused on targets having their own radioligand ( $D_2$ ,  $5\text{-HT}_{2A}$ ,  $5\text{-HT}_{1A}$ ,  $5\text{-HT}_4$ , and  $5\text{-HT}_6$  receptors), with competition experiments at increasing doses of loxapine.

The main results of our study can be summarized as: (1) loxapine succinate has in vitro a high affinity for the  $D_2$  receptor and  $5\text{-HT}_{2A}$  receptor with a  $5\text{-HT}_{2A}/D_2$  ratio superior to 1; (2) these in vitro results are confirmed in nonhuman primates with PET radiopharmaceuticals, by a high displacement potency loxapine for  $D_2$  receptors, and  $5\text{-HT}_{2A}$  receptors at low concentrations; (3) the in vivo PET study revealed that loxapine has possibly a significant affinity for other serotonergic receptors ( $5\text{-HT}_{1A}$  and  $5\text{-HT}_6$ ), which can be related to the clinical profile this compound.

All antipsychotics have actions at dopamine  $D_2$  receptors. "Atypical" ones, with a more loosely binding than dopamine and a dissociation constant higher than dopamine to these receptor (Seeman, 2002), may have a different action compared with "typical" on it. In addition, they block  $5\text{HT}_2$  receptors (Stahl, 2003), and the ratio of blockade of compounds of this class goes from

0.07 for clozapine to >100 for fluphenazine (Horacek et al., 2006). In clinical practice, this combination of  $5\text{-HT}_2$  with  $D_2$  blockade is related to the lower induction of extrapyramidal side effects but also to the effect observed on negative symptoms (Stahl, 2003).

With a  $5\text{-HT}_{2A}/D_2$  ratio >1, loxapine is shown to be close to an "atypical" profile, since its  $5\text{-HT}_{2A}$  occupancy is higher than its  $D_2$  occupancy. This study also demonstrates that loxapine succinate presented intermediate affinity for the  $D_1$ ,  $D_4$ ,  $D_5$ , and  $5\text{-HT}_{2C}$  and a nonsignificant affinity toward  $D_3$ ,  $5\text{-HT}_{1A}$ ,  $5\text{-HT}_4$ ,  $5\text{-HT}_6$ , and  $5\text{-HT}_7$  receptors. These results are in accordance with earlier preclinical studies describing its pharmacological action involving strong dopamine  $D_2$  receptor antagonism in addition to blocking activity at  $D_1$  and serotonin-2 ( $5\text{-HT}_2$ ) receptors as well (Singh et al., 1996). These binding results must be discussed. A high level of  $5\text{-HT}_2$  occupancy is not a sufficient condition for "atypicality" (in its clinical aspect). If atypical antipsychotic action is predicated on a combination of  $5\text{-HT}_2$  and  $D_2$  effects, then it requires >80%  $5\text{-HT}_2$  occupancy in conjunction with <80%  $D_2$  occupancy. Threshold  $D_2$  occupancy for EPS seems higher (84%) for olanzapine than others. Antipsychotic drugs with intrinsic anticholinergic activity may be associated with an upper threshold for EPS (Uchida et al).

As shown in a recent review publication (Popovic et al. 2015), loxapine at 10 to 100 mg/d was found to be equipotent at blocking  $D_2$  and 5-HT<sub>2A</sub> receptors.

Another contribution of our study is the use of PET radiopharmaceuticals. Our study is the first to provide PET autoradiographies on nonhuman primate brain tissue for loxapine. Nonhuman primate tissue is close to the neuroanatomy and the receptology found in human subjects (Phillips et al., 2014). With respect to  $D_2$  activity, a competition occurred at low concentrations of loxapine on  $D_2$  receptors. In a similar manner, the displacement of [<sup>18</sup>F]-altanserin (5-HT<sub>2A</sub> receptors) was significant and constant (−30 %) from 10 nM to 100 nM. Regarding the 5-HT<sub>2A</sub> receptors, they are blocked at low dosages like most atypical antipsychotic drugs (Seeman, 1981). Loxapine's superior affinity for 5-HT<sub>2</sub> (vs  $D_2$ ) observed in vitro was not found in our PET study. A previous PET study in schizophrenic patients found comparable results with a  $D_2$  receptor occupancy from 43% to 90% and a 5-HT<sub>2</sub> receptor occupancy from 27% to near saturation (Kapur et al., 1997). These discrepancies between in vivo and in vitro results can be explained by the higher affinity of the 7-hydroxyloxapine, a loxapine metabolite, for  $D_2$  receptors.

Another factor to be considered in the interpretation of the results is linked to the dosage of the molecule. According to PET studies in patients, antipsychotic threshold occupancy of  $D_2$  for antipsychotic action is about 65% for both “typical” and “atypical” antipsychotic drugs, regardless of whether 5-HT<sub>2A</sub> receptors are blocked or not, and extrapyramidal side effects occurs at about 80% of  $D_2$  receptor occupancy (Seeman, 2002). These in vivo data cannot be directly reproduced in our in vitro experimental conditions, despite the fact we used the same clinical radiopharmaceuticals. In other words, the possible significant affinity of loxapine for 5-HT<sub>1A</sub> and 5-HT<sub>6</sub> receptors we observed in primate brain tissue, and which was not measured in cellular cultures, has to be confirmed in vivo using the same radiotracers. Dosages used have an important impact on receptor spectrum; selection of an antipsychotic must take into account these binding profiles to ensure the antipsychotic effect at low doses and avoid the known adverse events (Correll et al., 2010). According to the literature and our results, at low doses (<100 mg/d), loxapine can be considered as “atypical” (5-HT<sub>2A</sub>/ $D_2$  ratio). At higher doses, the loxapine profile is more typical (strong  $D_2$  blocking). The potential antidepressant effect (5-HT<sub>1A</sub>/5-HT<sub>6</sub> antagonism) of loxapine needs additional clinical investigations.

Several methodological drawbacks and experimental limitations must be highlighted. Firstly, we must be careful before extrapolating the results of the binding study according to the fact that recombinant human cells (CHO, HEK-293, and GH4) overexpress the receptors in nonphysiological conditions. For example, binding results obtained for 5-HT<sub>1A</sub> and 5-HT<sub>6</sub> receptors in the cellular model were not in accordance with those obtained in the nonhuman primate brain tissue. The concentrations of ligand should also be considered, because the specific connections of a given ligand is saturable as it corresponds to a definite number of receptors. Additionally, the activities of the metabolites are not taken into account. Secondly and despite its preclinical uses, the PET imaging has well known limitations. All brain receptors of interest cannot be explored in vivo because of the currently limited number of PET radiopharmaceuticals (Zimmer and Luxen, 2012). If PET in vitro autoradiography in nonhuman primates is a potent translational method, it does not reproduce the in vivo production of metabolites (e.g., amoxapine, a loxapine's metabolite with a significant 5-HT<sub>6</sub> receptor affinity). Finally, extrapolation of in vitro loxapine concentrations to in vivo brain concentrations is difficult. If competition

studies with PET autoradiography can provide important data about loxapine affinity and binding properties toward receptors, only in vivo PET studies can measure the relation between the dose, the real receptor occupancy, and the clinical effect, all in a longitudinal manner (Zimmer and Luxen, 2012).

## Perspectives

The practical application of pharmacological knowledge in clinical practice has become an important issue of optimizing patients' treatment. Individualized treatment approaches need to consider current symptoms, comorbid conditions, as well as pharmacological aspects of medications (Correll et al., 2010). Our experimental approach focuses only on the pharmacological characteristics (and not on clinical issues resulting from the therapeutic effects). This approach is current since the recent new classification of psychotropic drugs focused on the brain targets (and not on the psychiatric indications).

To date, first line treatment for psychotic disorders is second-generation (“atypical”) antipsychotics. For decades, in clinical practice, loxapine has been a commonly used antipsychotic. However, its pharmacological mode of action was not yet been clearly established. Is loxapine a “typical” or an “atypical” antipsychotic? Historically classified as a typical antipsychotic, loxapine differs from these first-generation antipsychotics. Loxapine's receptor binding is equipotent in blocking 5-HT<sub>2</sub> and  $D_2$  receptors. Thus, in the Neuroscience Based Nomenclature classification (Zohar et al., 2015), according to its receptor affinity profiles, loxapine can be classified as a DS-RAN (Receptor antagonist  $D_2$ , 5-HT<sub>2</sub>) with chlorpromazine, iloperidone, lurasidone, olanzapine, paliperidone, sertindole, thioridazine, ziprasidone, and zotepine. Our study will help, providing a better understanding of loxapine's pharmacological domains of action. Taking into account our results, further studies of loxapine active metabolites (8-hydroxyloxapine and 7-hydroxyloxapine) and a dose-ranging study will be conducted.

## Conclusion

This article is an update of the dopamine-serotonin receptor binding characteristics of loxapine, a molecule that has a particular place between “typical” and “atypical” antipsychotics. This approach is needed and is in accordance with the newly proposed psychotropic drug reclassification (Zohar et al., 2015). At low doses, loxapine is shown to be in vitro close to an atypical profile, since its 5-HT<sub>2A</sub> binding occupancy is higher than its  $D_2$  binding (high 5-HT<sub>2A</sub>/ $D_2$  ratio). Our study also emphasizes the role of other targets ( $D_4$ ,  $D_5$  receptors). Widely used in acute situations at high dose, there is need for further PET studies exploring long-term prescriptions.

## Acknowledgments

The authors thank F. Bonnefoi, F. Liger, and S. Fieux for their technical assistance in radiochemistry and autoradiography. 2FNQ1P precursor was kindly provided by Dr T. Billard.

## Statement of Interest

F. Ferreri has received consulting fees within the last 3 years from Bristol Myers Squibb, E. Lilly and Company, Eisai, Lundbeck, and Otsuka pharmaceutical. D. Drapier discloses personal fees for travel reimbursement from EISAI, Janssen Lundbeck, and Otsuka not related to this study; personal fees from EISAI, Janssen,

Lundbeck, Otsuka not related to this study; and personal fees from EISAI related to this study. E. Baloche and M. Ouzid are Eisai SAS employees. L. Zimmer has received consulting fees within the last 3 years from Otsuka Pharmaceutical and Shire and research funding from Eisai, Neurolix, Novartis, Biocodex, Erytech, Pragma Therapeutics, and ORA Radiochemistry and Siemens. P. M. Llorca discloses personal fees for travel reimbursement from EISAI, Janssen Lundbeck, and Otsuka not related to this study; personal fees from EISAI, Janssen, Lundbeck, and Otsuka not related to this study; and personal fees from EISAI related to this study.

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