Is There a Role for GPCR Agonist Radiotracers in PET Neuroimaging?

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Positron emission tomography (PET) is a molecular imaging modality that enables in vivo exploration of metabolic processes and especially the pharmacology of neuroreceptors. G protein-coupled receptors (GPCRs) play an important role in numerous pathophysiological disorders of the central nervous system. Thus, they are targets of choice in PET imaging to bring proof concept of change in density in pathological conditions or in pharmacological challenge. At present, most radiotracers are antagonist ligands. In vitro data suggest that properties differ between GPCR agonists and antagonists: antagonists bind to receptors with a single affinity, whereas agonists are characterized by two different affinities: high affinity for receptors that undergo functional coupling to G-proteins, and low affinity for those that are not coupled. In this context, agonist radiotracers may be useful tools to give functional images of GPCRs in the brain, with high sensitivity to neurotransmitter release. Here, we review all existing PET radiotracers used from animals to humans and their role for understanding the ligand-receptor paradigm of GPCR in comparison with corresponding antagonist radiotracers.

Keywords: radiopharmaceutical, receptor, agonist, Positron emission tomography (PET), neuroimaging

INTRODUCTION

From Agonist Molecules to PET Radiopharmaceuticals

Positron emission imaging is a molecular imaging modality that enables exploration of metabolic processes in vivo. It uses specific radiotracers for specific molecular targets (Van de Bittner et al., 2014). The radiotracer must have several characteristics (Halliday et al., 2001; Pike, 2009; Honer et al., 2014): i.e., a specific binding to the target of interest with an acceptable signal-to-noise ratio, a passage through the BBB, and limited radiometabolites in the brain. The most

Abbreviations: µ, opioid receptors, mu opioid receptors; mGlu₁, metabotropic glutamate receptor 1; ¹¹C, carbon-11; ¹⁸F, Fluor-18; ¹TCM, one-tissue compartmental model; ²TCM, two-tissue compartmental model; ⁵-HT, serotonin; ⁵-HT₁A, subtype 1A of serotonin receptors; ⁵-HT₁B, subtype 1B of serotonin receptors; ⁵-HT₂A, subtype 2A of serotonin receptors; ⁵-HT₃, subtype 3 of serotonin receptors; ⁶-OH-DA, 6-hydroxy-dopamine; A₁, Receptor, subtype 1 of adenosine receptors; A₂, Receptor, subtype 2 of adenosine receptors; Ach, acetylcholine; AChEI, acetylcholinesterase inhibitors; AD, Alzheimer’s disease; APOE-e4, apoenzyme 4; BₚND, available density of receptors; BBB, blood–brain barrier; Bmax, maximal density of receptors; BP, binding potential; BPND, non-displaceable binding potential; BPP, plasma binding potential; CB₁, subtype 1 of cannabinoid receptors; CNS, central nervous system; D₂, type 2 dopaminergic receptors; D₃, type 3 dopaminergic receptors; E₃, maximal effect; GABA, γ-aminobutyric acid; GABAA, subtype A of GABA receptors; GABAB, subtype B of GABA receptors; GPCR, G-protein-coupled receptors; GTP, guanosine triphosphate; H₂, receptors subtype 3 of histamine receptors; IC₅₀, half-maximal inhibitory concentration; Kᵣ/ᵢ, affinity of receptors; Kᵦ, knock-out; LSD, diethylamide lysergic acid; M₁R, type 1 of muscarinic receptors; M₂R, type 2 of muscarinic receptors; M₃R, type 3 of muscarinic receptors; M₄R, type 4 of muscarinic receptors; nor-BNI, norbinaltorphimine; PET/fMRI, Positron emission tomography/function magnetic resonance imaging; PET, Positron emission tomography; PgP, P glycoprotein; SRTM, simplified tissue reference model; σ₁, sigma 1 receptors.
common molecular targets in PET neuroimaging are neurotransmitter receptors or transporters. PET imaging visualizes various neuroreceptors that can be located in vivo on presynaptic and/or post-synaptic sites, using a microdose of radioligand (i.e., tracer dose). PET imaging is therefore a powerful tool to demonstrate changes in neurotransmission in various CNS disorders, and can be used translationally in both animal models and humans. In addition to its contribution to the understanding of pathophysiological processes, PET imaging plays an important role in CNS drug development. It enables measurement of the proportion of receptors occupied by pharmacological doses of drugs of interest, in competition with a suitable radioligand specific to the same target. It can thus demonstrate brain penetration and in vivo binding to the target, which can be correlated to plasma concentrations to predict the effective dose range for clinical studies. They collect important information about the bioavailability of the drug candidate and contribute to the demonstration of brain penetration. Microdosing and drug occupancy studies have been shown to be very valuable for optimizing the development of drugs targeting the CNS. Another important application of PET neuroimaging is to measure in vivo fluctuations in endogenous neurotransmitter release. According to the occupancy model, the binding potential of a given radiotracer is modulated by the local concentration of the endogenous neurotransmitter in competition for the same receptors when the affinities of radioligand and neurotransmitter are in the same order of magnitude (Laruelle, 2000). All these applications rely on the development and full characterization of specific radiotracers. Consequently, although PET imaging provides interesting in vivo approaches to understanding neuropharmacology, it is currently limited by a lack of specific radiotracers for many known brain receptors. Moreover, the large majority of available radiotracers are antagonists and, as will be explained below, may not provide information about GPCR functional status in vivo; this fact, often disregarded, may be an important limitation for the interpretation of numerous clinical PET studies and probably explains certain controversies still ongoing in the field. The present review will therefore focus on the few agonist radiotracers that are currently available, highlighting their potential interest in PET neuroimaging, especially in humans in the form of radiopharmaceuticals.

The Pharmacological Paradigm of G Protein-Coupled Receptor Agonism

In vitro studies on membrane homogenates distinguished different properties in GPCR agonists and antagonists. While agonists bind to receptors with a single affinity, antagonists show two different affinities: high affinity for receptors coupled to G-proteins, and low affinity for uncoupled receptors (Figure 1). As reviewed recently (Shalgunov et al., 2019), these findings were demonstrated for various GPCRs: adrenergic (Hoffman and Lefkowitz, 1980), dopaminergic (Sibley et al., 1982; Leff et al., 1985), serotonergic (Battaglia et al., 1984; Gozlan et al., 1995; Watson et al., 2000) muscarinic (Ehlert, 1985), cannabinoid (Gullapalli et al., 2010), and opioid receptors (Law et al., 1985). The pharmacology of high-affinity receptors features specific phenomena: negative GTP cycle feedback loop, oligo-heterodimerization, internalization. While many in vitro studies revealed dysregulation of the balance between coupled and uncoupled states in these receptors, the implications for neurological disorders remains to be demonstrated, and new tools are needed. At present, few GPCRs can actually be studied using both antagonist and agonist radiotracers. Thus the different functional states of the receptors in vivo cannot be distinguished, making it impossible to specifically study the receptors in the high-affinity state which must represent the true (synapse) responsivity of the system to endogenous neurotransmission. Given that agonists bind preferentially to coupled receptors, PET imaging could disclose the active state of a receptor population.

Antagonists Are Often Used in PET Neuroimaging

The primary reason was that radiochemists and radiopharmacologists had access to a larger choice of antagonist molecules, initially developed as neuropharmacological tools; but there are also other reasons. Firstly, in terms of pharmacology, it is simpler to use an antagonist, which has only one affinity for a given receptor, as it does not discriminate between different subpopulations but rather provides an image of the global density of receptors. In contrast, using an agonist complicates the analysis due to its dual affinity for both high- and low-affinity receptors, providing an image with lower signal-to-noise ratio due to the lower density of the targeted receptors. Moreover, agonists are more likely to induce undesirable side effects for the patient if the quantity injected is too great (i.e., higher than a tracer dose): the specific activity needs to be high enough for a microdose of agonist to be injected, at subpharmacological level. Another problem is that the rate of conversion from high- to low-affinity state can cause the agonist to dissociate from its receptor too quickly: after stimulation by the agonist, the receptor is likely to switch to the low-affinity state within seconds (Mathis et al., 1997). Although dissociation is slower than the conversion from high- to low-affinity state, this can cast doubt on the functional state of the receptors actually targeted by the agonist (Seeman, 2012). All these reasons explain why PET neuroimaging using agonist radiotracers is considered challenging, as it raises a number of difficulties relating to radiopharmacy (e.g., the potential pharmacological effects of the radioligand if the specific affinity is too low), or to pharmacological concepts that are not at present fully elucidated, such as the conformational model of GPCR signaling itself. In fact, GPCRs can be considered as either pre-coupled to their respective G proteins or not (De Lean et al., 1980; Kent et al., 1980; Mongeau et al., 1992), or as initially non-coupled and interacting with G proteins after agonist stimulation (Laruelle, 2000; Skinbjerg et al., 2010).

Agonist Radiotracers to Explore the Coupling of GPCRs

On the other hand, the fact that neuroimaging mainly uses antagonist radiotracers incurs a number of limitations, in terms of both neurophysiology and pathophysiology. For...
neurophysiological exploration, the lack of sensitivity to endogenous neurotransmitter level or exogenous agonists at pharmacological dose, as will be discussed below, calls for the use of agonist radioligands. The physiological impact of GPCR functional state plays a major role in maintaining cellular homeostasis and effective neurotransmission. Compared to ion channels, which produce relatively simple and quick responses after stimulation by a ligand, GPCR signaling involves complex signaling cascades with production of numerous secondary messengers, interaction with various channels (Reboreda et al., 2018) and phosphorylation of diverse proteins, which may have varying long-term effects in the cell. Therefore, GPCRs are of critical functional importance in the CNS and are one of the most important drug targets in pharmacology, and especially neuropharmacology (Albizu et al., 2010; Björk and Svenningsson, 2011; Jastrzebska et al., 2011; Hauser et al., 2017). The same GPCR can interact with multiple signaling pathways that may vary across the brain [e.g., 5-HT_{1A} receptors (la Cour, 2006)]. For example, according to the recent concept of biased agonism, each ligand may preferentially stimulate a few of the numerous pathways that can interact with a GPCR. This may explain the diverse pharmacological effects observed for a given class of GPCR ligands (Albizu et al., 2010). The complexity of GPCRs needs to be studied more extensively in vivo, and requires the development of suitable tools such as agonist radiotracers and, ultimately, biased agonists. Pathophysiologically, G protein signaling is strongly involved in many neurodegenerative or neuropsychiatric disorders (Schreiber and Avissar, 2000; Avissar and Schreiber, 2006; Thathiah and De Strooper, 2011; Heese, 2013). Numerous in vitro studies showed that GPCR coupling state is modified in pathological conditions (Schreiber et al., 2009; Becker et al., 2014; Vidal et al., 2016). The exploration of coupled and non-coupled populations of receptors in vivo could be a key point in developing new therapies, monitoring treatment effects and identifying treatment responders. At present, it is difficult to explore G protein coupling in the brain in vivo due to a lack of non-invasive tools; it is measured either on brain slices in vitro (Pejchal et al., 2002; Shen et al., 2002) or in peripheral cells such as leukocytes (Golan et al., 2011).

According to in vitro pharmacological findings, agonist radiotracers may provide new tools, challenging the standard ligand receptor model in pharmacology (Laruelle, 2000). They seem to be involved in GPCR activity and reflect the responsiveness of the synapse signaling system (Shalgunov, 2017). Therefore, agonist radioligands could provide precise in vivo pharmacology by imaging only active neuroreceptors (Zimmer, 2016). PET would thus seem to be the means to shed light on GPCR properties in vivo.

STATE OF THE ART OF EXISTING PET AGONIST RADIOTRACERS FOR NEUROIMAGING

The following section comprises an exhaustive review of PET radiotracers with a translation to first in human are presented (Figure 2 and Table 1). See Supplementary Material for exhaustive review.

Dopaminergic Receptors

The dopaminergic system has five formally described subtypes of receptors (D_{1}, D_{2}, D_{3}, D_{4}, D_{5}). The dopaminergic system has benefited from the development of a large number of PET radiotracers. The majority of these radiotracers are derived from the many pharmacological tools and drug candidates that have been developed for psychiatry and then neurology. Among these, the use in humans of three agonist radiotracers of D_{2}/D_{3}R, allowed to investigate sensitivity to neurotransmitter release and estimate the proportion of coupling in comparison with the reference antagonist radiotracer, [^{11}C]raclopride.

D_{2}/D_{3} Receptors

[^{11}C]NPA

Ernst (1967) reported that apomorphine interacted with dopamine receptors. This observation launched intensive research about apomorphine’s structure/activity relationship, to define precisely the pharmacophore enabling dopamine
FIGURE 2 | Chemical structures of current agonist radiotracers of GPCRs. Dopamine receptors: (1) $^{[1]}$C-SKF 82957, (2) $^{[1]}$C-SKF79670, (3) $^{[1]}$C-SV-III-130, (4) $^{[1]}$C-5-OH-DPAT, (5) $^{[18]}$F-5-OH-FPAT, (6) $^{[18]}$F-FBu-AMC15 and derivatives, (7) $^{[18]}$F-ET-AMC15 and derivatives, (8) $^{[18]}$F-AMC20, (9) $^{[1]}$C-NPA, (10) $^{[1]}$C-MNPA, (11) $^{[18]}$F-MCL-524, (12) $^{[1]}$C-PHNO; serotonin receptors: (13) $^{[1]}$C-CUMI-101, (14) $^{[18]}$F-F15599, (15) $^{[18]}$F-F13714, (16) $^{[18]}$F-F13640, (17) $^{[1]}$C-Cimbi-5, (18) $^{[1]}$C-Cimbi-36, (19) $^{[18]}$F-FEDimbi-36; histamine receptors: (20) $^{[1]}$C-MK-8278; cannabinoid receptors: (21) $^{[1]}$C-OMAR or $^{[1]}$C-JHU75528, (22) $^{[18]}$F-MK-9470, (23) $^{[1]}$C-MePP, (24) $^{[1]}$C-CB-119, (25) $^{[1]}$C-PipSB, (26) $^{[18]}$F-PipSB, (27) $^{[1]}$C-SO5024; acetylcholine receptors: (28) $^{[18]}$F-TZTP; Opioïd receptors: (29) $^{[1]}$C-carfentanil, (30) $^{[1]}$C-PFO, (31) $^{[1]}$C-GR103352, (32) $^{[1]}$Cbuprenorphine; Sigma 1 receptors: (33) $^{[1]}$C-SA4503.
Table 1: Current agonist radiotracers of GPCRs with at least preclinical validation.

| Receptor | Subtype | No. | Radiotracer | Properties | PV | PA | CV | CA |
|----------|---------|-----|-------------|------------|----|----|----|----|
| Dopamine | D<sub>1</sub> | 1 | [<sup>11</sup>C]SKF 82957 | Agonist | + | + | − | − |
|          |         | 2 | [<sup>11</sup>C]-SKF76770 | Agonist | + | + | − | − |
| D<sub>2</sub>/D<sub>3</sub> | 3 | [<sup>11</sup>C]SV-III-130 | Partial agonist | + | + | − | − |
| D<sub>2</sub>/D<sub>3</sub> | 4 | [<sup>11</sup>C]-OH-DPAT | Agonist | + | − | − | − |
| D<sub>2</sub>/D<sub>3</sub> | 5 | [<sup>3</sup>H]5-OH-FPPAT | Agonist | + | − | − | − |
| D<sub>2</sub>/D<sub>3</sub> | 6 | [<sup>3</sup>H]FBU-AMC13 and derivatives | Agonists | + | − | − | − |
| Serotonin | 5-HT<sub>1A</sub> | 7 | [<sup>11</sup>C]CUMI-101 | Partial agonist | +/− | − | − | − |
| 5-HT<sub>1A</sub> | 8 | [<sup>3</sup>H]F2515599 | Biased agonist | + | − | − | − |
| 5-HT<sub>1A</sub> | 9 | [<sup>3</sup>H]F2513714 | Biased agonist | + | − | − | − |
| 5-HT<sub>1A</sub> | 10 | [<sup>3</sup>H]F213640 | Agonist | + | − | − | − |
| Histamine | H<sub>3</sub> | 11 | [<sup>11</sup>C]MK-8278 | Inverse agonist | + | + | + | + |
| Cannabinoid | CB<sub>1</sub> | 12 | [<sup>11</sup>C]OMAR or [<sup>13</sup>C]JHIL75528 | Inverse agonist | + | + | + | + |
| CB<sub>1</sub> | 13 | [<sup>11</sup>C]MK-9470 | Inverse agonist | + | + | + | + |
| CB<sub>1</sub> | 14 | [<sup>11</sup>C]MePPPEP | Inverse agonist | + | + | − | − |
| CB<sub>1</sub> | 15 | [<sup>11</sup>C]CB-119 | Inverse agonist | + | − | − | − |
| CB<sub>1</sub> | 16 | [<sup>11</sup>C]PipISB | Inverse agonist | + | − | − | − |
| CB<sub>1</sub> | 17 | [<sup>3</sup>H]F2PipISB | Inverse agonist | + | − | − | − |
| CB<sub>1</sub> | 18 | [<sup>11</sup>C]SD5024 | Inverse agonist | + | − | − | − |
| Acetylcholine | M<sub>2</sub> | 19 | [<sup>3</sup>H]FP-TZTP | Agonist | + | + | + | + |
| Opioid | μ<sub>1</sub>/μ<sub>2</sub> | 20 | [<sup>11</sup>C]carfentanil | Agonist | + | + | + | + |
| κ & δ | 21 | [<sup>11</sup>C]MEPEP | Agonist | +/− | − | − | − |
| κ | 22 | [<sup>11</sup>C]GR103345 | Agonist | + | − | − | − |
| all | 23 | [<sup>11</sup>C]Suprenorphine | Partial agonist | + | − | − | − |
| Sigma | σ<sub>1</sub> | 24 | [<sup>11</sup>C]SA46503 | Agonist | + | + | + | + |

PV: preclinical validation; PA: preclinical applications; CV: clinical validation; CA: clinical applications.

Translation to human (Green)/Only preclinical studies (Blue).

receptor binding/agonism/stimulation. [<sup>11</sup>C]Apomorphine itself was synthesized and evaluated in rat brain as a radioligand of D<sub>1</sub>-like and D<sub>2</sub>-like receptors (Zijlstra et al., 1993; Finnema et al., 2010). Brain uptake and specific binding ratios were too low for satisfactory application in PET imaging. Hwang et al. (2000) proposed [<sup>11</sup>C] radiolabeling of NPA, first synthesized in Neumeyer et al. (1973). NPA is a D<sub>2</sub> agonist with higher affinity for D<sub>2</sub> receptors at high affinity than for D<sub>3</sub> receptors at low affinity (Sibley et al., 1982). Previous studies demonstrated that its tritiated analog crossed the BBB, with high uptake in striatum (van der Werf et al., 1983). Hwang and colleagues described the radiosynthesis of [<sup>11</sup>C]NPA, and biodistribution studies in rodents confirmed high uptake in striatum and a high striatum/cerebellum ratio (3.4 at 30 min post-injection). Haloperidol pretreatment decreased the striatum/cerebellum ratio to 1.3 at 30 min in rat brain. PET imaging studies on a single baboon also revealed a high striatum/cerebellum ratio of 2.8 at 45 min post-injection. A blockade study with haloperidol strongly decreased the striatum/cerebellum ratio, confirming specific binding to D<sub>2</sub>/D<sub>3</sub> receptors in vivo. Cumming et al. (2002) demonstrated that [<sup>3</sup>H]NPA was more sensitive to endogenous dopamine in striatum than the antagonist [<sup>11</sup>C]raclopride in a study on living mice: both dopamine depletion by reserpine and dopamine increase by amphetamine had greater effects on the binding potential of tritiated NPA. A study in anesthetized Göttingen miniature pigs, using compartmental analyses, showed fast tracer metabolism, compensated by high brain uptake during the first minutes; the striatal binding potential was comparable with [<sup>11</sup>C]NPA binding in vivo (Cumming et al., 2003). Another quantitative study in baboon validated the use of model-based approaches to quantify [<sup>11</sup>C]NPA binding in vivo (Hwang et al., 2004).
Other PET studies in baboons confirmed its higher sensitivity to dopamine release in striatum compared to $[^{11}\text{C}]$raclopride (Narendran et al., 2004; Hwang et al., 2005). The proportion of D$_2$ receptors at high affinity was estimated to be 79% in the striatum using a bolus plus constant infusion (Narendran, 2005). A study in 1 male baboon did not show significant difference between time of recovery after amphetamine-induced dopamine release as measured by $[^{11}\text{C}]$raclopride and $[^{11}\text{C}]$NPA (Narendran et al., 2006). Subsequent studies focused on translation to humans. An initial study concluded that administration of a common dose of radiotracer (370 Mbq) yielded an acceptable dosimetric range in all organs (Laymon et al., 2009). Reproducibility studies and kinetic modeling in a second human study confirmed that $[^{11}\text{C}]$NPA was a reliable tool to image D$_2$/D$_3$ receptors in high-affinity state in striatum (Narendran et al., 2009). Narendran and colleagues performed a comparative evaluation of $[^{11}\text{C}]$NPA and $[^{11}\text{C}]$raclopride to measure amphetamine-induced dopamine release in the human striatum after oral amphetamine pretreatment: decreases in BP$_{ND}$ level were slightly greater for the agonist radiotracer, whereas no significant difference was found for BP$_{P}$ (Narendran et al., 2010). McCormick et al. (2010), in ex vivo studies, demonstrated that isoflurane increased the binding and amphetamine sensitivity of $[^{11}\text{C}]$NPA and other agonists in comparison with $[^{11}\text{C}]$raclopride, which may have been a confounding factor in several preclinical studies that reported higher sensitivity of agonist radiotracers to endogenous dopamine.

An initial clinical study in cocaine abusers versus controls did not find any differences in D$_2$/D$_3$ binding in striatum, contrary to several studies that previously reported lower binding of $[^{11}\text{C}]$raclopride in cocaine abusers. The authors concluded that D$_2$/D$_3$ receptors in high-affinity state may be unaltered in cocaine dependence (Narendran et al., 2011). Other preclinical studies were performed using $[^{11}\text{C}]$NPA: one ex vivo study demonstrated that it was more effective than $[^{11}\text{C}]$raclopride in detecting an increase in receptor availability following unilateral injections of 6-OH-DA in rat, a classical model of Parkinson’s disease (Palner et al., 2011); another study in rat suggested differential distribution of tritiated NPA and raclopride in the striatum, with comparable B$_{max}$ in the dorsal striatum and lower B$_{max}$ for $[^{3}\text{H}]$NPA in the ventral striatum (Minuzzi and Cumming, 2010).

$[^{11}\text{C}]$MNPA

MNPA, a methoxy-NPA derivative, was initially described in Gao et al. (1990), and was characterized as a D2R agonist (K$_{i} = 0.17$ nM). A preliminary PET study in cynomolgus monkey in Halldin et al. (1992) demonstrated high binding of $[^{11}\text{C}]$MNPA in the striatum. Finnema et al. (2005) described an optimized radiosynthesis and further in vivo PET experiments in cynomolgus monkey. The study confirmed previous findings about D$_2$ specificity: pretreatment with unlabeled raclopride considerably decreased the signal in regions known to contain D$_2$ receptors. Two advantages were mentioned in comparison with $[^{11}\text{C}]$NPA: a fivefold higher affinity, which might enable quantitative analysis in extrastriatal regions such as thalamus, and easier radiosynthesis of $[^{11}\text{C}]$MNPA, which only needs $[^{11}\text{C}]$methylation. Metabolite analysis did not show any lipophilic metabolites which could interfere with the parent-compound signal in the brain. The sensitivity of the radiotracer to synaptic dopamine levels was then compared versus $[^{11}\text{C}]$raclopride in cynomolgus monkeys; $[^{11}\text{C}]$MNPA showed higher sensitivity. Using the same methodology of high-affinity state quantification proposed by Narendran with $[^{11}\text{C}]$NPA, the authors suggested that about 60% of D$_2$ receptors were in the high-affinity state (Seneca et al., 2006). In preparation for future human studies, a kinetic brain analysis and whole-body imaging study was performed in monkeys (Seneca et al., 2008a). Brain distribution volumes were identified using a 2-tissue compartment model and in accordance with the known distribution of D$_2$/D$_3$ receptors, and the estimated dosimetry in human, extrapolated from the preclinical results, was moderate to low. However, the authors reported that BP$_{ND}$ values of $[^{11}\text{C}]$MNPA were lower than $[^{11}\text{C}]$raclopride or other agonist radiotracers such as $[^{11}\text{C}]$PHNO and $[^{11}\text{C}]$NPA, probably due to higher uptake in the cerebellum. Seneca et al. (2008b), the same team conducted a PET study in rat using a bolus/infusion paradigm following dopamine depletion pretreatment with reserpine plus alpha-methyl-para-tyrosine. Dopamine occupancy at baseline was estimated at 53% in rat brain, in agreement with previous results with other agonist radiotracers. In the same study, binding in striatum was placeable with raclopride but not by BP897, a selective D$_3$ ligand, suggesting that $[^{11}\text{C}]$MNPA binds specifically to D$_2$ and not D$_3$ receptors. Skinbjerg et al. (2009) conducted a precise pharmacological characterization of MNPA with in vitro studies on recombinant cells and membrane preparations. They concluded that MNPA was a full agonist of D$_2$ and also D$_3$ receptors (in contrast to previous in vivo findings). Also, two high- and low-affinity binding states were observed only for membrane preparations and not for cells. Other preclinical studies were conducted the same year: Finnema et al. (2009) determined the occupancy of the agonist apomorphine at increasing doses in cynomolgus monkey using $[^{11}\text{C}]$raclopride and $[^{11}\text{C}]$MNPA. Contrary to the hypothesis that agonist radioligands are more sensitive than antagonists to competition with pharmacological doses of agonists, there was no difference between Ki and ID50 determined with both radiotracers, suggesting that all D$_2$ receptors are in high-affinity state or that there is only a single receptor state. Tokunaga et al. (2009) reported an example of a drug discovery approach using in vivo imaging with $[^{11}\text{C}]$MNPA to detect dopamine neurotransmission system modulation by MPEP, an antagonist of group mGlu$_2$ receptors. Steiger et al. (2009), an optimization of radiosynthesis was also described, with a time of about 40 min after radionuclide production.

The first clinical trial, on 10 subjects, in Otsuka et al. (2009), used a classical protocol with arterial blood sampling and metabolite analysis (and PET procedure). Results showed a distribution pattern in concordance with the D$_2$ receptor distribution. The SRTM and transient equilibrium methods were validated to estimate binding potentials. Another clinical study investigated binding of the antipsychotic risperidone on high- or low-affinity D$_2$ receptors with $[^{11}\text{C}]$raclopride and $[^{11}\text{C}]$MNPA and found that risperidone bound indifferently to both states of
D₂ receptors, with comparable occupancies and ED50 values for both tracers (Kodaka et al., 2010). In 2013, a study investigated the reproducibility of the binding potential ratio of [¹¹C]MNPA to [¹¹C]raclopride, reflecting the proportion of receptors in high-affinity state compared to overall receptor density; reproducibility was satisfactory in the caudate and putamen (Kodaka et al., 2012). More recently, the same team studied the different D₂ receptor affinity states in 11 antipsychotic-free schizophrenic patients, using [¹¹C]raclopride and [¹¹C]MNPA; the binding potential ratio (agonist/antagonist) was significantly higher in the putamen in patients than control subjects, despite unchanged levels of total D₂ receptors (Kubota et al., 2017).

Other preclinical studies using [¹¹C]MNPA were conducted over the years. Skinbjerg et al. (2010) showed that in vivo striatal binding of the tracer was unchanged in dopamine beta-hydroxylase-deficient mice, concluding that their increased sensitivity to psychostimulants was not due to a higher proportion of receptors in the high-affinity state. The same year, the D₂ receptor occupancies of quinpirole, aripiprazole, and haloperidol were estimated in conscious rats, using tritiated MNPA, PHNO, and raclopride (Peng et al., 2010). All compounds showed similar occupancy values with the different radioligands, presumably due to a high proportion of receptors in the high-affinity state in vivo. Another study, focusing on stress in conscious monkeys, showed that stress level correlated negatively with [¹¹C]raclopride binding, and positively with [¹¹C]MNPA binding (Tsukada et al., 2011).

[¹¹C]PHNO

The first radiolabeling and preclinical evaluation of the naphthoxazine derivative D₂-agonist (+)-4-Propyl-3,4,4a,5,6,10b-hexahydro-2H-naphtho[1,2-b]-[1,4]-oxazin-9-ol, or (+)-PHNO, was reported in Wilson et al. (2005). [¹¹C]-(+)-PHNO binding in rat brain was highly selective and specific to D₂ receptors. The tracer also showed sensitivity to increases and decreases in endogenous dopamine levels. The full D₂ agonistic properties of (+)-PHNO were previously documented (Jones et al., 1984). The first-in-man study was performed 1 year later (Willeit et al., 2006). [¹¹C]-(+)-PHNO displayed good brain uptake and favorable kinetics; test–retest data suggested BP estimates to be reliable, and pre-treatment with haloperidol reduced specific binding without detectable changes in cerebellum, validating its utility as a D₂-receptor agonist radioligand for PET. Binding in the globus pallidus was greater than with the D₂ antagonist [¹¹C]raclopride, suggesting that [¹¹C]-(+)-PHNO also binds significantly to D₃ receptors in humans. Tracer distribution, displaceability by endogenous dopamine, specificity and modeling properties were further explored in cat and compared versus [¹¹C]raclopride (Ginovart et al., 2006a). Although Scatchard analysis showed comparable Bmax values with both [¹¹C]-(+)-PHNO and [¹¹C]-raclopride, the agonist was more sensitive than the antagonist to dopamine release (BP inhibition up to 83 versus 56%, respectively). The signal-to-noise ratio in the striatum was also 2.5-fold higher than that of [¹¹C]NPA. Unusually high binding in the globus pallidus was reported in baboons and explained by higher affinity of [¹¹C]-(+)-PHNO for D₃ receptors than other D₂/D₃ radioligands, possibly contributing to its greater vulnerability to endogenous dopamine (dopamine affinity being higher for D₃ than D₂ receptors) compared to other radioligands (Narendran et al., 2006).

Kinetic modeling of the tracer was then described in humans (Ginovart et al., 2006b), to enable [¹¹C]-(+)-PHNO binding to be quantified in clinical studies. Willeit et al. (2008), the D₂–D₃ agonist radioligand was reported to be sensitive to competition with endogenous dopamine in humans. Several studies were then performed with [¹¹C]-(+)-PHNO, to study the high-affinity state of D₂ receptors, D₃ receptors and endogenous dopamine release in schizophrenia, addiction or according to social status (Graff-Guerrero et al., 2008; Mizrachi et al., 2011, 2012; Le Foll et al., 2013; Matuskey et al., 2015; Caravaggio et al., 2016). This radiotracer was often compared to [¹¹C]raclopride, but it is unclear if the differences between the two radiotracers are due to the agonistic properties of [¹¹C]-(+)-PHNO or to its higher affinity for D₃ receptors. For instance, [¹¹C]-(+)-PHNO shows preferential uptake in the ventral striatum and globus pallidus, due to the high density of D₂ receptors in these areas, whereas [¹¹C]raclopride shows preferential uptake in the dorsal striatum; [¹¹C]-(+)-PHNO wash-out is also slower in the globus pallidus compared to the other regions (Graff-Guerrero et al., 2008). Similarly, a PET study in Parkinson’s disease showed a significant decrease in [¹¹C]-(+)-PHNO levels in the globus pallidus, in contrast to [¹¹C]raclopride, and an agonist/antagonist ratio that decreased proportionally to motor deficit and lowered mood, interpreted as the consequence of D₁ receptor density modifications (Boille et al., 2009). Searle et al. (2010) suggested that [¹¹C]-(+)-PHNO uptake is due to high-affinity D₂ receptors in the dorsal striatum, to high-affinity D₂ receptors and D₃ receptors in the ventral striatum, globus pallidus and thalamus, and only to D₃ receptors in the substantia nigra). Numerous preclinical studies were also performed using [¹¹C]-(+)-PHNO. Seeman et al. (2007) reported a 2-to-3-fold increase in high-affinity D₂ receptors in rat following repeated injection of amphetamine, explaining why the animals were more sensitive to dopaminergic agonists. One year later, the sensitivity was compared between [¹¹C]-(+)-PHNO and [³H]raclopride after various pharmacological challenges in conscious rats (McCormick et al., 2008). Similar degrees of inhibition were shown for both radiotracers following the pre-injection of amphetamine, cold NPA (a full agonist), aripiprazole (a partial agonist), haloperidol and clozapine (D₂ antagonists). However, these results were contradicted by another study that showed greater ex vivo inhibition of [³H]PHNO binding by NPA than by [³H]raclopride, and greater displacement of the agonist radiotracer in amphetamine-sensitized rats (Kumar et al., 2006; Seeman, 2009). Contrasting results were published the same year by McCormick et al. (2009), underlining significant discrepancies in the field which require further study.

D₄ Receptors

Although no convincing radiotracer specific to D₄ receptors is presently available for PET neuroimaging in the human brain, the discovery of an inverse agonist was reported in Prante et al. (2008). The compound was derived from FAUC 113 and FAUC 213 and was more selective for D₄ receptors than for D₂
and D₃ receptors. The [¹⁸F]-labeled molecule showed specific binding in rat brain in vitro, but no further investigations have yet been reported.

Serotonergic Receptors
Serotonin neurotransmission is characterized by a large number of subfamilies of receptors (14 are currently described). After the dopaminergic system, it is the second neurotransmission system to have benefited from the development of many PET radiotracers. While a significant number of them can be used in humans as radiopharmaceuticals, most are antagonists and currently very few agonists are available. However, the therapeutic potential of these many sub-families of receptors in psychiatry and neurology justifies further research in this area.

5-HT₁₄ Receptors
From [¹⁸F]F15599 to [¹⁸F]F13640
Much effort has been made to develop a radiotracer agonist of 5-HT₁₄ receptors, with initially limited success. These attempts included exploration of derivatives of various structures (analogs of 8-OH-DPAT and apomorphine, arylpiperazine, or attempts included exploration of derivatives of various structures) and currently very few agonists are available. However, the PET radiotracers. While a significant number of them can be shown high affinity (Kᵢ = 2.2 nM) and excellent specificity for 5-HT₁₄ receptors, acting as a full agonist both in vitro and in pharmacological tests in rats, with preferential activity at post-synaptic receptors (Newman-Tancredi, 2011). Despite encouraging in vitro results, the signal-to-noise ratio was insufficient for PET imaging. Its structural analog, [¹⁸F]F13714, displaying higher affinity for 5-HT₁₄ receptors (Kᵢ = 0.1 nM), was also evaluated (Lemoine et al., 2012). Despite a better SNR and evidence of binding to 5-HT₁₄ receptors in vivo, [¹⁸F]F13714 binding was irreversible in rat, cat and rhesus monkey, which suggests it would be difficult to quantify binding parameters in humans. Interestingly, [¹⁸F]F13714 was also compared with the antagonist [¹⁸F]MPPF in conscious and anesthetized marmosets; it displayed a markedly different distribution pattern from [¹⁸F]MPPF, with highest uptake in raphe and cortical areas, as opposed to hippocampus and amygdala for the antagonist radiotracer. It also showed region-specific uptake changes in isoflurane-anesthetized animals, contrary to [¹⁸F]MPPF for which global increase throughout the brain was observed (Yokoyama et al., 2016). Top design a radiotracer that would be easier to quantify, the structural analog [¹⁸F]F13640 was recently evaluated (Vidal et al., 2018). F13640 also exhibits high selectivity for 5-HT₁₄, but intermediate affinity (Kᵢ = 1 nM) compared to the previous two attempts. [¹⁸F]F13640 showed specific binding to 5-HT₁₄ receptors and agonistic properties in vitro and in vivo in rats, cats and rhesus monkeys, despite a distribution pattern contrasting with antagonist radiotracers (and similar to that of [¹⁸F]F13714). Moreover, ex vivo autoradiography experiments using pharmacological challenge with d-fenfluramine in rats suggested that [¹⁸F]F13640 is far more sensitive to competition with endogenous serotonin than the antagonist [¹⁸F]MPPF. Despite a slow washout, tracer binding is reversible, with increased washout after administration of fenfluramine, a serotonin releaser, during scanning in rats and cats (unpublished data). An autoradiographic study performed on postmortem hippocampus slices from AD patients at different Braak stages also demonstrated a decrease in [¹⁸F]F13640 binding in the CA1 area, occurring earlier than the decrease in [¹⁸F]MPPF binding in AD subjects. Further studies are ongoing to characterize the properties of [¹⁸F]F13640, and a first-in-man study is currently underway (clinicaltrials.gov number: NCT03347331). The first images in human showed a binding pattern different from that seen with the conventional antagonist 5-HT₁₄ radiopharmaceutical [¹⁸F]-MPPF (Colom et al., 2019).

5-HT₂₄ Receptors
From [¹¹C]Cimbii to [¹¹C]Cimbii
Ettrup et al. (2010) reported [¹¹C]-labeling and evaluation of the N-benzyl phenylethylamine derivative Cimbi-5, previously described as a selective and very potent agonist for 5-HT₂₄ receptors (Braden et al., 2006). [¹¹C]Cimbi-5 distribution was consistent with the known 5-HT₂₄ distribution, and it was blocked by the antagonist ketanserin in pig brain (Ettrup et al., 2010). In an attempt to optimize the target-to-background binding ratio, the same team evaluated 9 other phenylethylamine analogs of [¹¹C]Cimbi-5 in pig (Ettrup et al., 2011). The analog [¹¹C]Cimbi-36 was identified as the most promising candidate for PET imaging of 5-HT₂₄ receptors as it showed the highest target-to-background binding ratio and was displaceable by ketanserin. In vitro studies confirmed that Cimbi-36 was a potent and selective 5-HT₂₄ agonist (Kᵢ = 1 nM, ED₅₀ = 0.5 nM). Its properties were then characterized in non-human primate and compared with the antagonist [¹¹C]MDL-100907 (Finnema et al., 2014). [¹¹C]Cimbi-36 distribution was again consistent with the known 5-HT₂₄ distribution and blocked by ketanserin in all brain regions except the cerebellum, which was found to be a suitable reference region. Binding potential was approximately half that of [¹¹C]MDL-100907 across cortical regions but higher in other brain regions such as the choroid plexus, which was found to be related to 5-HT₂₃ receptor binding as it was blocked by the 5-HT₂₃ ligand SB 242084. The authors concluded that [¹¹C]Cimbi-36 was an agonist radioligand suitable for examination of 5-HT₂₄ receptors in cortical regions and of 5-HT₂₃ receptors in the choroid plexus of the primate brain. The first-in-man study was performed in 29 healthy volunteers, with arterial input measurement and pretreatment with ketanserin in 5 subjects (Ettrup et al., 2014). The authors concluded that [¹¹C]Cimbi-36 was a suitable agonist radioligand for PET imaging of high-affinity 5-HT₂₄ receptors in the cortex, and that cerebellum was an appropriate reference tissue for quantification without blood sampling. Recently, test-retest reproducibility was investigated and the distribution was compared to the antagonist [¹⁸F]altanserin in humans (Ettrup et al., 2016).
The results showed excellent test–retest reproducibility and a high correlation between the two radiotracers, Cimbi-36 and altanserin, except in regions with high 5-HT2C receptor density (choroid plexus and hippocampus), where [11C]Cimbi-36 binding is probably to both 5-HT2A and 5-HT2C receptors. Sensitivity in detecting changes in endogenous 5-HT levels was also explored in pig brain using various pharmacological challenges, by simultaneous measurement of extracellular 5-HT concentration with microdialysis and PET imaging (Jorgensen et al., 2017). There was a significant correlation between 5-HT levels and 5-HT2A occupancy, indicating that [11C]Cimbi-36 is sensitive to competition with serotonin, although only at sufficiently high release. Another study in rhesus monkey demonstrated significant decrease in [11C]Cimbi-36 binding in most brain regions following administration of fenfluramine at 5 mg/kg (Yang et al., 2017). The tracer was found to be more sensitive to 5-HT release than the antagonist [11C]MDL 100907, and with sensitivity comparable to [11C]AZ10419369, a 5-HT1B partial agonist currently considered to be one of the most sensitive radioligands.

Finally, a study in humans recently compared two positions of [11C]-labeling for the tracer and concluded that the position initially chosen in the previous studies produced a higher signal-to-noise ratio (Johansen et al., 2018).

**Cannabinoid Receptors**

The endocannabinoid system is more recent in discovery compared to previous monoaminergic systems. This neurotransmission system seems to play key modulatory roles in the brain and much effort has been made to try to understand precisely its pathophysiological role in various behavioral and neurological diseases. While there are currently two known subtypes of cannabinoid receptors, termed CB1 and CB2, only the first have benefited from the development of agonist radiotracers.

**CB1 Receptors**

[11C]JHU75528 or [11C]OMAR

Fan et al. (2006) reported the synthesis of [11C]JHU75528, to image CB1 receptors. The tracer showed higher in vitro affinity and lower lipophilicity than the two prototypical CB1 agonists, rimonaban and AM281. Autoradiography studies in mice and PET studies in baboon showed high cerebral uptake, good SNR and specific binding displaced by the cold ligand or rimonaban pretreatment. Metabolite analysis demonstrated that a few fractions cross the BBB (Horti et al., 2006). The first clinical assays on humans confirmed a good CB1 receptor quantification (Horti, 2007; Wong et al., 2008). In terms of quantification, plasma reference graph analysis (Logan et al., 1990) was found to be more reliable than the two-compartmental model for estimating Vt. It was not possible to use pons or white matter as reference regions, due to small size and lack of favorable kinetics, respectively (Wong et al., 2010). Comparison between healthy volunteers and schizophrenic patients found elevated values in patients, especially in the pons region. This preliminary study showed the potential of Vt values to prove CB1 involvement in schizophrenia and to predict the type and severity of clinical symptoms. Gao et al. (2012) proposed a new synthesis route for [11C]OMAR and analogs, and Normandin explored more precisely the quantification of the tracer in humans (Normandin et al., 2015). They found that multilinear analysis was the most robust method. Test–retest reproducibility was satisfactory. There were significant sex differences in tracer properties, and especially in metabolism and brain uptake. These findings suggest that [11C]OMAR is a reliable radiotracer and that, in this case, gender differences must be considered in PET analysis.

[18F]MK-9470

Continuing previous efforts to develop a CB1 receptor radiotracer, Burns et al. (2007) developed [18F]MK-9470, a specific inverse agonist, in a context of drug development. The in vitro affinity of MK-9470 was 0.7 nM with a 60-fold selectivity for CB1 receptors in comparison with CB2 receptors. Autoradiography studies on rhesus brain slices showed a signal consistent with CB1 receptor distribution. PET studies in monkeys showed rapid uptake with displaceable binding by the specific inverse agonist MK-0364. In vivo PET study in humans showed slow kinetics with high uptake in striatum, frontal cortex and posterior cingulate. Metabolite analysis and test–retest reproducibility were satisfactory enough to envisage [18F]MK-9470 as a suitable radiotracer to explore CB1 receptor density. These findings were applied to determine the occupancy of the inverse agonist MK-0364. One year later, a biodistribution and radiation dosimetry study demonstrated acceptable dose exposure and the feasibility of multiple scans (Van Laere et al., 2008b). The tracer was also used to assess gender differences in CB1 receptor distribution and changes in receptor expression with healthy aging (Van Laere et al., 2008a). Several clinical studies were then performed, including a drug occupancy study of the CB1 receptor inverse agonist taranabant (Addy et al., 2008), and studies exploring the relationship between CB1 receptors and personality traits (Van Laere et al., 2009), temporal lobe epilepsy (Goffin et al., 2011), Parkinson’s disease (Van Laere et al., 2012), eating disorder (Gérard et al., 2011; Ceccarini et al., 2016), migraine (Van der Schueren et al., 2012), schizophrenia (Ceccarini et al., 2013), AD (Ahmad et al., 2014), chronic cannabis use (Ceccarini et al., 2015), alcohol abuse (Ceccarini et al., 2014), and Huntington’s disease (Ceccarini et al., 2019). The tracer was also used in numerous preclinical studies (Goffin et al., 2008; Casteels et al., 2010a,b,c,d, 2011, 2014; Gérard et al., 2010; Ooms et al., 2014; Cleeren et al., 2018). Several studies were also performed to optimize quantification in humans (Sanabria-Bohórquez et al., 2010) and rats (Casteels et al., 2012; Miederer et al., 2018) and radiosynthesis (Thomae et al., 2014).

**Muscarinic Receptors**

Muscarinic acetylcholine neurotransmission have been described for several decades and its interest has resurfaced more recently because of their implications in neurodegenerative diseases, justifying research work in PET neuroimaging. Five subtypes
of muscarinic receptors have been determined, named M1–M5. Among them, only the M2 family has benefited from the development of agonist PET radiotracers used in humans.

**M2 Receptors**

[^18F]FP-TZTP

In this context, Sauerberg et al. (1992) developed a series of muscarinic agonists containing a thiadiazolyl moiety. Based on these data, three fluorinated derivatives of TZTP were evaluated in vitro for their affinity toward the various muscarinic receptors (Kiesewetter et al., 1995). The derivative FP-TZTP displayed higher affinity for M2 than M1 receptors (K_i = 2.2 vs. 7.4 nM) and was radiolabeled with [^18F] and further evaluated in rat.[^18F]FP-TZTP displayed specific binding, being dose-dependently blocked by the analog P-TZTP, also a M2-prefering agonist, but only partially blocked by antagonists of M1 or M2 receptors.[^18F]FP-TZTP was then evaluated in preclinical in vitro and in vivo studies in rats and monkeys: [^18F]FP-TZTP showed specific binding to M2 receptors, significantly inhibited by cold compound and L-687,306, a muscarinic agonist. Uptake was significant in cortical and subcortical regions, with low uptake in cerebellum. Metabolism study in rats showed no significant presence of radiometabolites in the brain (Kiesewetter et al., 1999). The 1-compartmental model was the most reliable for determining distribution volume in rhesus monkey (Carson et al., 1998).

Finally, [^18F]FP-TZTP was sensitive to variations in ACh levels induced by physostigmine, an AChEi. Kiesewetter et al. (2003) reported 1-step automated radiosynthesis of [^18F]FP-TZTP, and Ma et al. (2003) described a method using liquid–liquid PE and solid phase extraction to rapidly measure concentrations of tracer and metabolites thanks to the previous identification of the metabolite structures by LC-MS-MS (Ma et al., 2002).

The first-in-man study was performed in Podruchny et al. (2003) on healthy young and older volunteers. The binding pattern of [^18F]FP-TZTP was consistent with the known M2 receptor distribution. Older subjects had significantly greater distribution volumes, which was explained by lower synaptic acetylcholine concentrations. Jagoda et al. (2003) used different models of KO mice to confirm the M2 selectivity of [^18F]FP-TZTP, demonstrating a significant decrease in binding (from 51 to 61%) only in M2R KO mice, almost none in M1R KO mice (about 20% in amygdala and hippocampus), and none in M3R and M4R KO mice. Considering the fact that P-TZTP and the cold agonist FP-TZTP used in competition studies could produce changes in cerebral blood flow, decreasing the PET signal by reduced tracer delivery rather than by competition for receptors, Shimoji et al. (2003) showed that inhibition of [^18F]FP-TZTP by these compounds was not due to agonist-induced reduction in CBF: the degree of tracer uptake inhibition was unchanged when a peripheral muscarinic antagonist was combined with muscarinic agonists to prevent the CBF changes induced by agonists alone. In a new clinical study, [^18F]FP-TZTP was used to compare two populations of healthy subjects with and without apolipoprotein E-epsilon 4 allele, which is associated with increased susceptibility to AD and age-related memory problems (Cohen et al., 2003). APOE-epsilon4+ subjects had greater distribution volumes in gray matter than APOE-epsilon4- subjects, which was again interpreted in terms of synaptic acetylcholine concentration differences. [^18F]FP-TZTP was then used to understand the cholinergic contribution to the emotional and sensory effects of procaine. Procaine dose-dependently decreased [^18F]FP-TZTP specific binding (Benson et al., 2004). Following the 2003 clinical study, a new study was performed to evaluate the influence of age and APOE-epsilon4 genotype on the increase in acetylcholine concentration induced by physostigmine infusion and the distribution volumes of [^18F]FP-TZTP (Cohen et al., 2006). It was also demonstrated that physostigmine induced a decrease in [^18F]FP-TZTP uptake, and that both age and APOE-e4 genotype influenced the modulation of PET signal by physostigmine infusion. Furthermore, [^18F]FP-TZTP was used to demonstrate the involvement of M2 receptors in mood disorders: there was decreased binding in patients suffering from bipolar disorder, which could be due to a reduction in M2 receptor density or affinity, or to an increase in endogenous acetylcholine levels (Cannon et al., 2006). Van Oosten et al. (2009) reported an optimized radiosynthesis using a new precursor. Cannon et al. (2011), another clinical study was performed combining [^18F]FP-TZTP-PET and genetic analyses: it was shown that single nucleotide polymorphisms for the M2R gene were associated with changes in [^18F]FP-TZTP binding in bipolar disorder patients. Finally, Ravasi et al. (2012) found that constant infusion of [^18F]FP-TZTP was better than bolus injection for performing microPET in rodents: blood clearance and metabolism were too rapid to measure a reproducible input function after bolus injection.

**Histaminergic Receptors**

There are four known histamine receptors, H1, H2, H3, and H4. The first imaging works focused on the H1 receptor but without the development of PET agonists. More recently, H3 receptor, a target with emerging pathophysiological implications, has led to the development of agonist radiotracers.

**H3 Receptors**

The H3 receptor has a presynaptic location and is involved in the regulation of histamine neurotransmission and modulation of release of other neurotransmitters (Van Laere et al., 2013). Thus, it has been demonstrated that, instead of only interfering with the negative feedback loop of histamine like an antagonist, H3 inverse agonists potentiate histaminergic neurotransmission by decreasing constitutive H3 signaling. These pharmacological properties suggest new treatments for various psychiatric or neurodegenerative diseases. According to the two-state model of agonist action, inverse agonists may have higher affinity for the inactive state of the receptor (Leff et al., 1985; Berg and Clarke, 2018). Concomitant development of H3 receptor inverse agonist radiotracers therefore seems important for the development of new H3 inverse agonists as therapeutic agents, as such radiotracers may better reflect the population of receptors actually targeted by these new ligands.
Spiro-Isobenzofuranone Derivative: $[^{11}\text{C}]$MK-8278

In this context, in Hamill et al. (2009) reported the radiosynthesis and evaluation of two promising inverse agonists, as shown by the inhibition of basal $[^{35}\text{S}]$GTPgammaS binding to membrane homogenates expressing recombinant $\text{H}_3$ receptor derived from a family of spiro-isobenzofuranone-based compounds (Jitsuoka et al., 2008). The study described a radiosynthesis with high specific activity and revealing appropriate in vitro autoradiographic distribution in rhesus monkey and human brain, and specific binding in PET experiments in rhesus monkey for both compounds, with greater brain uptake for $[^{11}\text{C}]$MK-8278. Using a bolus plus infusion method and in vivo PET imaging with $[^{11}\text{C}]$MK-8278 in rhesus monkeys, the authors also determined the occupancy of diverse $\text{H}_3$ receptor inverse agonists in relation to their plasma concentration. Van Laere et al. (2014) confirmed the utility of $[^{11}\text{C}]$MK-8278 as a specific radioligand to evaluate in vivo occupancy of new $\text{H}_3$ inverse agonists in human brain.

They first described whole-body biodistribution and dosimetry in humans, and found that the effective dose was in the typical range of other $[^{11}\text{C}]$-labeled radiopharmaceuticals. The binding parameters of $[^{11}\text{C}]$MK-8278 were quantified using a metabolite-corrected arterial input function. 1TCM and SRTM methods, considering pons as a reference region, showed reproducible estimates of $V_t$ and $B_{P,AD}$ values, respectively. Finally, the authors determined the human pharmacological profile of two inverse agonists, MK-024 and MK-3134, taken orally at various doses 6 h prior the PET scan; they thus obtained the receptor occupancy of both compounds as a function of oral dose or plasma concentration, demonstrating the key role of $[^{11}\text{C}]$MK-8278 for characterizing target engagement of $\text{H}_3$ inverse agonists (by calculating RO as a function of plasma concentration).

**Opioid Receptors**

There are four major subtypes of opioid receptors named delta ($\delta$), kappa ($\kappa$), mu ($\mu$), and nociceptin receptors. Agonistradiotracers of opioid receptors development was mainly derived by radiolabeling of existing drugs and displayed extensive use to understand physiopathological mechanisms in various diseases.

**$\mu$ Opioid Receptors**

$[^{11}\text{C}]$Carfentanil

Radiosynthesis of the very potent $\mu$-opioid agonist $[^{11}\text{C}]$carfentanil was reported in Dannals et al. (1985), quickly followed by a first PET study in humans and baboons (Frost et al., 1985). High radioactivity levels were found in the striatum and thalamus and low levels in the cerebellum and occipital cortex, consistent with the known regional density of $\mu$ receptors. $[^{11}\text{C}]$Carfentanil binding was also strongly reduced by pretreatment with the antagonist naltrexone, confirming its high specificity and suitability as an opioid receptor agonist radiotracer. It was then used in a clinical PET study to demonstrate elevated $\mu$ receptor concentration in temporal lobe epilepsy (Frost et al., 1988). A multicompartamental analysis was performed to quantify the binding parameters of $[^{11}\text{C}]$carfentanil in human brain (Frost et al., 1989), and an in vitro binding study with the tritiated molecule further demonstrated its selectivity for the $\mu$ receptor subtype in human and rat brain (Titeler et al., 1989).

Frost et al. (1990), the binding patterns of $[^{11}\text{C}]$carfentanil and the antagonist $[^{11}\text{C}]$diprenorphine were compared in humans, showing different regional distributions that were explained by the non-selectivity of diprenorphine for the different subtypes of opioid receptors. In addition, a study focusing on temporal epilepsy demonstrated significant changes in opioid receptors with $[^{11}\text{C}]$carfentanil but not $[^{11}\text{C}]$diprenorphine (Mayberg et al., 1991). Zubieta et al. (1996), a study demonstrated the involvement of the opioid system in addiction by showing that $[^{11}\text{C}]$carfentanil binding was increased in cocaine-dependent subjects compared to healthy controls, and correlated positively with cocaine craving. Since then, a huge number of clinical PET studies of $\mu$ receptors have been performed with $[^{11}\text{C}]$carfentanil, focusing on epilepsy (Madar et al., 1997), the menstrual cycle (Smith et al., 1998), gender and age differences (Zubieta et al., 1999), addiction (Zubieta et al., 2000; Benchéris et al., 2004; Gorelick et al., 2005, 2008; Heinz et al., 2005; Greenwald et al., 2007; Scott et al., 2007a; Weerts et al., 2008, 2011, 2014; Ghizta et al., 2010; Ray et al., 2011; Falcone et al., 2012; Minkowski et al., 2012; Mitchell et al., 2012; Wand et al., 2012; Kuwabora et al., 2014; Domino et al., 2015; Mick et al., 2016; Nuechterlein et al., 2016; Hermann et al., 2017; Majuri et al., 2017), eating disorders (Benchéris et al., 2005; Karlsson et al., 2015, 2016; Tuominen et al., 2015; Jousta et al., 2018), PTSD (Liberson et al., 2007), major depression (Kennedy et al., 2006; Prossin et al., 2011; Hsu et al., 2015; Peciña et al., 2015a; Light et al., 2017), pain (Benchéris et al., 2002; Scott et al., 2007b, 2008; Wager et al., 2007; Harris et al., 2009; Dos Santos et al., 2012; Hagelberg et al., 2012; Campbell et al., 2013; Martikainen et al., 2013; DaSilva et al., 2014a,b; Peciña et al., 2015b; Karjalainen et al., 2017) and behavior or emotions (Hsu et al., 2013; Mitchell et al., 2013; Nummenmaa et al., 2015, 2018; Karjalainen et al., 2016; Manninen et al., 2017; Tuulari et al., 2017; Saanijoki et al., 2018).

$[^{11}\text{C}]$Carfentanil was used to measure the receptor occupancy of buprenorphine in heroin-dependent subjects (Zubieta et al., 2000; Greenwald et al., 2007), and of nalmefene in healthy subjects (Ingman et al., 2005). A multimodal study also evaluated $\mu$ receptor occupancy by the opioid receptor antagonist naltrexone and the inverse agonist GS1524198 in relation with the modulation of the fMRI response to a food stimulus (Rabiner et al., 2011). $[^{11}\text{C}]$Carfentanil appeared to be sensitive to endogenous opioid fluctuations in studies showing decreased binding potential during somatic pain (Benchéris et al., 2002; Scott et al., 2007b), after placebo administration (Zubieta et al., 2005; Scott et al., 2008) and after pharmacological challenge associated with release of opioid peptides (Colasanti et al., 2012).

$[^{18}\text{F}]$-labeled derivatives of carfentanil, $[^{18}\text{F}]$fluoro-pentyl carfentanil and the analog sufentanil, $[^{18}\text{F}]$fluoro-propyl-sufentanil were developed by Henriksen et al. (2005a). Both compounds had nanomolar affinity for $\mu$-opioid human receptors, and their distribution in rat brain slices was consistent with $\mu$-opioid receptor expression. The derivative of sufentanil...
produced almost no radioactive metabolites in mouse brain (Henriksen et al., 2005b). However, no further results have yet been reported.

κ Opioid Receptors

$[^{11}C]GR89696$ and $[^{11}C]GR103545$

$[^{11}C]GR89696$, a racemate that is an antagonist of $\kappa_1$ receptors and agonist of $\kappa_2$ receptors, was synthesized and evaluated in mice in Ravert et al. (1999). Uptake was in good agreement with known kappa opioid receptor distribution and was inhibited by kappa opioid-selective drugs. The R and S enantiomers of $[^{11}C]GR89696$ were later characterized separately, showing that only the R enantiomer $[^{11}C]GR103545$ exhibited selective and saturable binding to kappa receptors (Ravert et al., 2002). $[^{11}C]GR103545$ regional binding patterns in baboon brain were also consistent with the established distribution of kappa receptors, and binding was blocked by naloxone pretreatment (Talbot et al., 2005). Another study showed that $[^{11}C]GR103545$ also had high affinity for kappa receptors in humans in vitro ($K_i = 0.02 \text{ nM}$) and in awake rhesus monkeys (Schoutz et al., 2010). $K_d$ and $B_{max}$ were estimated using a Scatchard plot in a bolus/infusion protocol, in the same species (Tomasi et al., 2013).

The first-in-man study was performed in Naganawa et al. (2014) and showed the suitability of the tracer for imaging and quantifying kappa receptors in humans, although quantification of kinetic parameters can be difficult due to lack of a reference region and to slow kinetics. Recently, a pilot study of kappa opioid receptor binding in major depression was conducted, using $[^{11}C]GR103545$ to compare distribution volumes between healthy volunteers and patients suffering from major depressive disorder; no significant differences were detected (Miller et al., 2018). The tracer was also used to investigate the effect of various ligands on the kappa opioid receptor in rodents (Placzek et al., 2015). First, the authors validated the use of $[^{11}C]GR103545$ to measure drug occupancy at kappa receptors by showing that specific binding was blocked by pre-injection of GR89696 and the antagonists naloxone and LY2795050. Then, they showed that injections of the kappa receptor agonist salvinorin A 1 min before the PET scan induced a dose-dependent decrease in $[^{11}C]GR103545$ binding potential. At sufficiently high dose, this decrease persisted up to 2.5h after administration, although the half-life of salvinorin A is only few minutes, suggesting an agonist-induced adaptive response by kappa receptors. The same authors demonstrated that, although the agonist $[^{11}C]GR103545$ and the antagonist $[^{11}C]LY2459989$ have similar distribution patterns in rat brain, they differed in sensitivity to competition with various kappa receptor ligands (Placzek et al., 2018): the binding potential of both tracers was reduced to a similar extent by pre-injection of the opioid receptor antagonists naloxone and naltrrexone, and the selective kappa receptor antagonist LY2795050, whereas other kappa antagonists blocked $[^{11}C]GR103545$ binding more effectively (Bruchas et al., 2007). Finally, the kappa agonists butorphan and GR89696 showed comparable impact on the binding potentials of $[^{11}C]GR103545$ and $[^{11}C]LY2459989$, whereas the other agonists, salvinorin A and U-50488, significantly decreased $[^{11}C]GR103545$ uptake and had no effect on $[^{11}C]LY2459989$ (Placzek et al., 2018).

The authors explained these findings by a likely different conformation recognized by LY2459989, as the mutation of the residue D138 dramatically decreased the affinity of LY2459989 and not GR103545 for kappa opioid receptors.

Sigma Receptors

Initially considered as part of opioid receptors, pharmacological properties of sigma receptors identified them as a specific family of receptors. Two subfamilies of sigma receptors are currently identified, $\sigma_1$ and $\sigma_2$ receptors. If the role of $\sigma_1$ receptors is not well-defined, potential therapeutic applications emerge in experimental neurology, justifying the research of PET agonist radiotracers.

$\sigma_1$ Receptors

$[^{11}C]SA4503$

Kawamura et al. (1999) reported $[^{11}C]$-radiolabeling and evaluation of SA6298, a selective $\sigma_1$ receptor agonist. The compound showed high brain uptake in vivo in rats and 1 cat, but the signal was mostly non-specific. The same team evaluated the analog $[^{11}C]SA4503$, which has slightly lower affinity but better specificity for $\sigma_1$ receptors, with more encouraging results (Kawamura et al., 2000): there was high specific uptake in rat brain in vivo, as shown by blocking studies which decreased the signal proportionally to the $\sigma_1$ affinity of the various ligands. Moreover, no radiolabeled metabolites were found in the brain. $[^{11}C]SA4503$ binding in mouse and cat brain was also highly specific (Kawamura et al., 2000). Further experiments in conscious monkeys confirmed it as a promising radiotracer (Ishiwata et al., 2001). Although uptake increased continuously during control scans, tracer binding was displaced by haloperidol, which has high affinity for $\sigma_1$ receptors. $[^{11}C]SA4503$ was then used to investigate the time-course occupancy of $\sigma_1$ receptors by haloperidol in mice (Ishiwata et al., 2003) and humans (Ishiwata et al., 2006), and its tritiated analog was used to measure age-related changes in $\sigma_1$ receptor expression in rat brain in vitro (Kawamura et al., 2003a). The density of $\sigma_1$ receptors significantly increased with age, a finding that was confirmed in a PET study in monkeys (Kawamura et al., 2003b), but not in rat brain in vivo by Ramakrishnan et al. (2015), who showed a decrease in BP in several brain regions in aged rats.

Mishina et al. (2005), a clinical study compared $[^{11}C]SA4503$ binding in healthy volunteers and Parkinson's disease patients, and found no difference between controls and patients but a significant reduction in BP in the more injured side of the anterior putamen in patients, as assessed by $[^{11}C]$CFT binding. Quantitative analysis of $\sigma_1$ receptors in the human brain using $[^{11}C]SA4503$ was reported in Sakata et al. (2007). Another study was performed in AD patients: compared to elderly volunteers, AD patients had lower BP in the cortex and cerebellum (Mishina et al., 2008). The high occupancy of $\sigma_1$ receptors by the SSRI fluvoxamine and not by paroxetine (Ishikawa et al., 2007) and high occupancy by the AChEI donepezil (Ishikawa et al., 2009) were demonstrated in living human brain at therapeutic doses. Several fluorinated analogs of $[^{11}C]SA4503$ were synthesized and evaluated, including $[^{18}F]$FE-SA4503 (Elsinga et al., 2002, 2004), which is non-selective for the different subtypes of sigma...
receptor, $[^{18}F]$FE-SA5845, less favorable in terms of kinetics, and $[^{18}F]$FM-SA4503, which showed high specific binding and is more selective of $\sigma_1$ receptors (Kawamura et al., 2007).

**WHAT IS DIFFERENT WITH ANTAGONIST RADIOTRACERS?**

Here, we propose to discuss the *in vivo* differences between agonist and antagonist radiotracers. The initial concept supporting the use of agonists as radiotracers is based on their preference for the high-affinity state of GPCR receptors as opposed to the total population of receptors, as observed *in vitro*. This concept should be associated to obvious differences between agonists and antagonists, such as differential sensitivity to competition with various ligands, to pharmacological alterations of G-protein/receptor coupling and to pathological alterations. Although the issue is likely to be much more complex *in vivo*, and it has proved difficult to demonstrate the existence of different coupling states of GPCR receptors in living organisms, a number of studies did highlight the above-mentioned differences. In addition, some data even suggest distinct brain distribution patterns between agonist and antagonist radiotracers for certain GPCR.

**In vivo Binding of Agonists Versus Antagonists**

According to *in vitro* data, agonist radiotracers are expected to display lower specific binding and available receptor density than reference antagonist radiotracers. Some studies directly compared the BP ($B_{max}/K_d$) of an agonist and an antagonist radiotracer specific for the same target in the same subjects. For instance, Kodaka et al. (2012) compared the binding potentials of the $D_2/D_3$ receptor radiotracers $[^{11}C]$MNPA and $[^{11}C]$raclopride in two humans, showing that the agonist's binding potential was about four times lower than the antagonist's. The BP ratio between the two radiotracers was highly reproducible on test–retest, and was suggested as a possible estimate of the proportion of receptors in high-affinity state as compared to overall $D_2/D_3$R density. A similar approach was used for 5-HT$_{1A}$ receptors, using the partial agonist $[^{11}C]$CUMI-101 and the antagonist $[^{11}C]$WAY-100635 in non-human primates (Kumar et al., 2012). The authors reported an average 45% lower binding potential for $[^{11}C]$CUMI-101, with some regional variations (highest proportion of coupled receptors in the parahippocampal gyrus, and lowest in the amygdala and putamen). Another study in marmosets, comparing the full 5-HT$_{1A}$ agonist $[^{18}F]$FI513714 and the antagonist $[^{18}F]$MPF, showed that antagonist binding potential was approximatively threefold higher in 5-HT$_{1A}$R-rich regions (such as hippocampus and amygdala), whereas in striatum and thalamus BP$_{ND}$ levels were similar between the two tracers (Yokoyama et al., 2016). These regional variations in the proportion of 5-HT$_{1A}$R in high-affinity state were even greater in conscious animals. Taken together, these studies advocate differential targeting of GPCR receptors by agonists, which display lower binding potential likely because they bind preferentially to high-affinity receptor states. Therefore, if both radiotracers are available for a given GPCR, the proportion of highly effective receptors can be determined as compared to overall receptor density, in physiological or pathological conditions. However, interpretation of the above results is limited by a number of factors.

Firstly, comparison of binding potentials reflects the differences in $B_{max}/K_d$ ratio rather than $B_{max}$ directly; although the affinity of radiotracers is classically known from *in vitro* binding studies, the actual *in vivo* affinity can differ significantly. Unfortunately, very few studies directly compared the *in vivo* density of receptors targeted by an agonist versus an antagonist radiotracer. Using Scatchard analyses of PET data in 2 cats, Ginovart et al. (2006a) estimated the $B_{max}$ of the agonist $[^{11}C]$PHNO to be similar to that of $[^{11}C]$raclopride, casting doubt on differential binding of agonist/antagonist radiotracers *in vivo*. On the other hand, the $B_{max}$ of $[^{11}C]$NPA was shown to be about 79% of that of $[^{11}C]$raclopride in baboon (Narendran, 2005).

Another problem in comparing agonists and antagonists is the selectivity of the compounds: it is rather common for them not to be fully selective for a given GPCR, complicating the interpretation of results. This is precisely the case concerning $[^{11}C]$PHNO, which has higher affinity for the $D_3$R receptor subtype than other dopaminergic radiotracers (Narendran et al., 2006). Consequently, most clinical findings using this radiotracer were interpreted in terms of $D_3$R alterations rather than $D2/D3$R coupling state. Another example is the 5-HT$_{2A}$R agonist radiotracer $[^{11}C]$Cimbi-36, which displayed lower binding than the antagonist $[^{11}C]$MDL-100907 in cortical regions in rhesus monkey, but not in the hippocampus or choroid plexus, due to significant binding to 5-HT$_{2C}$ receptors (Finnema et al., 2014). In human brain, $[^{11}C]$Cimbi-36 provided BPs that were comparable to (in cortical regions) or higher than (in 5-HT$_{2C}$R-rich regions) the antagonist $[^{18}F]$altanserin (Ettrup et al., 2016). Estimated $B_{avali}$ Values (knowing the plasma protein binding of each tracer and the *in vitro* affinities of each ligand) were also similar. Finally, the partial µ opioid receptor agonist $[^{11}C]$carfentanil and the antagonist $[^{11}C]$diprenorphine were also compared in baboons (Shiue et al., 1991) and humans (Frost et al., 1990): the greater uptake of $[^{11}C]$diprenorphine in the striatum or cingulate cortex was explained by its significant affinity for other opioid receptor subtypes or different kinetic properties compared to $[^{11}C]$carfentanil. In this regard, it is obvious that direct comparison of agonist and antagonist radiotracers can also be hindered by large differences in the kinetic parameters $K_1$, $k_2$, $k_3$ and $k_4$, especially as different modeling approaches may be needed to quantify BP.

Considering the existence of two affinity sites for the agonist, the kinetics of displacement by endogenous neurotransmitters or exogenous drugs differs between agonist and antagonist radiotracers. This is the case for $[^{11}C]$raclopride and $[^{11}C]$NPA, where quantitative autoradiography showed biphasic displacement for the agonist and monophasic displacement for the antagonist with increasing concentration of LSD (Minuzzi and Cumming, 2010). This phenomenon introduces another degree of complexity in comparing antagonist and agonist displacement in pharmacological challenge. Furthermore, it was
demonstrated that activated 5-HT_{IA} receptors induced a specific dynamics on the cell surface in vivo, which can modify in vivo receptor distribution (Pucadyil and Chattopadhyay, 2007). This could explain the difference between agonist and antagonist radiotracers and also the frequent discrepancies between in vitro and in vivo data.

As the occupancy of a GPCR by its specific endogenous neurotransmitter is expected to be greater in the high-than in the low-affinity state, the estimated B_{max} value for an agonist may be closer to the B_{avail} value for an antagonist than the theoretical B_{max} values, which adds another level of complexity in comparing agonists versus antagonists. Therefore, considering the number of parameters that influence radiotracer binding quantification in vivo, it seems reasonable to conclude that it will generally be difficult to calculate directly the ratio of coupled receptors to total receptors density reliably enough to provide meaningful pathophysiological information. Likewise, simply comparing binding potentials or even B_{avail} between agonist and antagonist radiotracers in physiological conditions is unlikely to answer the question of the actual existence of a high-affinity GPCR state in vivo.

**Greater Sensitivity to Neurotransmitter Release?**

The dopamine system is the system most widely explored in terms of neurotransmitter release monitoring using PET (Finnema et al., 2015). Several PET radioligands of D_{2}/D_{3} receptors are sensitive to dopamine release, such as the benzamide derivative [^{11}C]raclopride, an antagonist that has been extensively used to evaluate changes in dopamine release in the striatum, providing new insights into the role of dopamine in pathological and physiological conditions. Other antagonists with higher D_{2} affinity, such as [^{11}C]FLB-457 and [^{18}F]fallypride, have been used to monitor extracellular dopamine fluctuations in extrastriatal regions where the density of D_{2} receptors is lower. In theory, the sensitivity of these radioligands is limited by the fact that endogenous dopamine preferentially targets the high-affinity state of D_{2}/D_{3} receptors, which is only a part of total receptor density as measured by antagonist radiotracers (Laruelle, 2000).

It was demonstrated that agonist radiotracers of D_{2}/D_{3} receptors such as [^{11}C]MNPA, [^{11}C]NPA and [^{11}C]PHNO were even more sensitive to DA release, both in animals (Ginovart et al., 2006a; Gallezot et al., 2014 for [^{11}C]PHNO; Narendran et al., 2004 for [^{11}C]NPA; Seneca et al., 2006; Skinbjerg et al., 2010 for [^{11}C]MNPA and humans Narendran et al., 2010; Shotbolt et al., 2012; Caravaggio et al., 2014). These experiments determined the proportion of high- and low-affinity states of D_{2}/D_{3} receptors by means of amphetamine challenge or Scatchard analysis (Table 1).

Of the numerous attempts to develop a radiotracer sensitive to serotonin release, only a few experiments with [^{11}C]CUMI-101 (Milak et al., 2011) ([^{18}F]F13640 (Vidal et al., 2018) and [^{11}C]Cimbi36 (Jorgensen et al., 2017; Yang et al., 2017) showed sensitivity. These difficulties suggest notable differences between dopamine and serotonin competition systems: degree of receptor availability, proportion of high-affinity state receptors, and size of the accessible receptor pool (Paterson et al., 2010). However, agonist radiotracers seem to be more appropriate than antagonist radiotracers to evaluate neurotransmitter release. For example, the literature does not report significant sensitivity for the 5-HT_{IA} receptor antagonist [^{18}F]MPPF, but only then in the case of a huge release of serotonin (Zimmer et al., 2002; Rbah et al., 2003), suggesting a small proportion of coupled receptors in basal state (Udo de Haes et al., 2006). Higher sensitivity to neurotransmitter release than for agonist radiotracers was also suggested in vivo for [^{11}C]GR103455 (for complete details, see Table 1). However, in pharmacological challenge paradigms, many agonist radiotracers lack direct comparison with antagonist radiotracers.

Finally, the effect of anesthesia has to be taken into account, particularly in preclinical studies. As observed by several teams, anesthesia is also responsible for changes in cerebral blood flow, receptor affinity and, finally, neurotransmission (Tsukada et al., 2002; Hassoun et al., 2003; Yokoyama et al., 2016). More precisely, it is also known to affect GPCR coupling (Seeman and Kapur, 2003). In vivo experiments on conscious subjects are consequently recommended, but assessment of such a protocol is not always possible in animals. Quick translation to human experiments is therefore desirable. Further investigation must be envisaged to explore the in vivo behavior of agonist and antagonist radioligands. This will certainly affect the current ligand-receptor paradigm.

**The Concept of Internalization**

Internalization is a phenomenon that is induced by agonist stimulation. Briefly, variations in the neurotransmitter, especially increasing levels in the synapse, can influence receptor crossing from cell surface to intracellular compartment. This has been demonstrated for dopamine, serotonin (Riad et al., 2001), muscarinic (Keith et al., 1998) mu opioid receptors (Quclh et al., 2014), and α_{2} receptors (Olli-Lähdesmäki et al., 1999). This adaptive process can interfere with the binding of agonist radiotracers, especially in pharmacological challenge, which induces a massive release of neurotransmitter into the synapse. Thus, the observed decrease in binding following pharmacological challenge could be due to internalization more than to direct competition (Zimmer et al., 2004; Ginovart, 2005). Consequently, the level of lipophilicity could explain differences in ligand binding: lipophilic radioligands bind both free and sequestered receptors, whereas hydrophilic ligands bind receptors only at the cell surface (Aznavour et al., 2006).

For example, it has been proven that, after amphetamine challenge, the acute effect of neurotransmitter release is responsible for a large decrease in the levels of both types of radiotracer (Narendran et al., 2004; Ginovart et al., 2006a; Seneca et al., 2006). In the case of [^{11}C]MNPA and [^{18}F]fallypride, an antagonist, amplitude was greater for the agonist. On a short time-scale, the phenomenon of displacement was dominant; then, on a longer scale, internalization caused incomplete recovery of both radiotracers (Skinbjerg et al., 2010). However, [^{18}F]fallypride is known to bind internalized receptors with affinity twofold lower than free receptors. Consequently, [^{18}F]fallypride was also affected by internalization. The process of internalization remains unclear: Narendran et al. (2004) found
no difference between NPA and raclopride in recovery time after amphetamine challenge.

In the case of 5-HT
\textsubscript{2A}
 receptors, Ettrup et al. (2010) found no differences in binding between the agonist \textsuperscript{[11]}C|Cimbi36 and the antagonist \textsuperscript{[18]}F|Psalanserin. They found a correlation between \textit{B}_\text{max} for both radiotracers. \textit{B}_\text{max} was almost the same (164 for the agonist and 173 for the antagonist). But these results were not in agreement with \textit{in vitro} data suggesting internalization of 80% of receptors.

### The Case of Biased Agonism

G protein-coupled receptors display two different states, an active state (coupled receptor) and an inactive state (non-coupled receptor), and it was demonstrated \textit{in vitro} that a given receptor may be coupled to different subtypes of G protein (Offermanns et al., 1994; Laugwitz et al., 1996) depending on its location in the brain (Jin et al., 2001). In this context, studies demonstrated that an agonist can selectively trigger a single transduction pathway among the numerous transduction pathways of GPCR (Berg and Clarke, 2006; Kenakin, 2015; Luttrell et al., 2015). Consequently, agonist ligands for a single target provide their own functional signature by selecting a specific transduction pathway. This biased agonism is related to allosteric modification of the receptor defined by multiple conformations, each depending on ligand interaction with signaling proteins (Berg and Clarke, 2006; Kenakin and Christopoulos, 2012). In this context, Yokoyama et al. (2016) compared agonist and antagonist radiotracers on 5-HT
\textsubscript{1A} receptors in non-human primates. The binding of the biased agonist \textsuperscript{[18]}F|F13714 was not only lower than \textsuperscript{[18]}F|MPPF but very different. Although images revealed binding all consistent with 5-HT
\textsubscript{1A} receptor distribution (cortical regions, amygdala, hypothalamus, and raphe nucleus), there were notable differences in intensity: e.g., lower in hippocampus and amygdala and higher in the cingulate and insular cortices for the agonist radiotracer. \textsuperscript{[18]}F|MPPF showed twofold higher binding in the hippocampus and amygdala. The authors attributed these differences to the biased agonism of F13714, interacting with specific G protein subtypes and targeting a specific brain region composed of presynaptic receptors: raphe striatum and thalamus. The notion of biased agonism was also recently explored by PET/MR imaging, and contributes to defining the existence of biased agonism on 5-HT
\textsubscript{1A} receptors (Vidal et al., 2018). In the kappa-opioid receptor, differences in the dynamics of receptor structure induced by the agonist \textsuperscript{[11]}C|GR103545 versus the antagonists \textsuperscript{[11]}C|LY2795050 and \textsuperscript{[11]}C|LY2459989 were used to explain the \textit{in vivo} discrepancies observed on PET imaging (Placzek et al., 2018). Biased agonism requires exploring different transduction pathways composed of a single receptor and is a key point in the hypothetical \textit{in vivo} differences between agonist and antagonist radiotracers. \textit{In vitro} data clearly show the existence of two different affinity states for GPCR, but there are still difficulties in demonstrating this on PET imaging, suggesting that there may be multiple receptor conformations rather than just two affinity sites. Going further, it was also demonstrated that some 5-HT
\textsubscript{2A} antagonists are able to trigger arrestin pathways and induce paradoxical desensitization of GPCR (Gray and Roth, 2001).

### WHAT IS THE ROLE OF PET AGONISTS IN NEUROIMAGING?

#### Improving the Measure of Endogenous Neurotransmitter Release

It is assumed from the \textit{in vitro} data that antagonists are less sensitive to neurotransmitter release than agonists. Agonists bind only to high-affinity state receptors, whereas antagonists bind to both high- and low-affinity state receptors equally. Therefore, when initiating competition, the antagonist is not effectively involved (Paterson et al., 2010; Finnema et al., 2015). On the other hand, agonist radiotracers provide direct estimation of the target affinity of endogenous neurotransmitters (Narendran et al., 2004). Considering pharmacological findings suggesting that agonists are more sensitive to neurotransmitter release, it is of great interest to test this hypothesis \textit{in vivo}. However, as seen before, it is difficult to demonstrate high sensitivity; experimental conditions are a determining factor. Measuring neurotransmitter release involves knowing the exact neurotransmitter level, by microdialysis. The endogenous levels are too low to be estimated at baseline with a radiotracer (Finnema et al., 2015), and it is necessary to perform pharmacological challenge to obtain huge neurotransmitter release. The development of modeling of neurotransmitter release contributes to understanding these mechanisms (Normandin et al., 2012).

#### Precision Pharmacology to Evaluate Neurologic Disorders and New Therapeutics

Agonist radiotracers are useful tools to develop new agonist drug candidates. In this context, it seems to be more appropriate to choose the same type of ligand when performing drug-occupancy studies. Also, in activation studies it is possible to visualize the impact of drug or task on the active population of receptors. Therefore, there is great interest in testing this clinically. Although this has not yet been formally demonstrated, it can be proposed that pathological conditions lead to a specific decoupling of GPCRs, leading to a functional deficit of neurotransmission. PET imaging by radiopharmaceutical agonists may enable precise definition of which GPCR pathways are damaged in CNS disorders and which pathways are still functional.

Firstly, it is possible to evaluate the difference in basal binding between control and pathologic conditions. Secondly, basal binding can contribute to therapeutic optimization of a drug and of early drug development by using the drug occupancy paradigm. However, for example, a study on cynomolgus monkeys found no differences in drug occupancy of amphetamine measured with \textsuperscript{[14]}C|raclopride or \textsuperscript{[14]}C|MNPA (Finnema et al., 2009). On the other hand, an \textit{ex vivo} study showed that the D\textsubscript{2} agonist NPA was more effective in detecting an increase in receptor availability in the early stages of Parkinson's disease. Likewise, concerning 5-HT
\textsubscript{1A} receptors in a postmortem study in Alzheimer patients, the radiolabeled agonist F13640 was more effective in detecting transient over-expression
of 5-HT<sub>1A</sub> receptors, followed by functional decoupling of these same receptors, before a decrease in their total density at a later Braak stage (Vidal et al., 2016).

In therapeutics, it could be possible to stimulate only specific pathways with biased agonists, to offset GPCR damage in brain. Thus, it appears that agonist radiotracers could be useful for developing precision pharmacology (Schaffhausen, 2017), with numerous applications in pharmacotherapeutics. In any case, it is now recommended to confirm these hypotheses with in vivo PET studies.

Future Challenges and Conclusion

Agonist radiotracers provide many opportunities to decipher the ligand-GPCR paradigm in neuropharmacology. It is possible to pursue in vitro findings with an in vivo design. The concomitant use of antagonist and agonist radiotracers sheds light on complex phenomena such as reversible conversion of high- to low-affinity receptors and internalization. However, the existence of two receptor affinity states has not yet been clearly demonstrated in vivo (Finnema et al., 2010; Skinbjerg et al., 2012). There are discrepancies between in vitro and in vivo findings, certainly because the in vivo environment of the neuron is complex. Study conditions are also a determining factor. Anesthesia or changes in blood flow modify radiotracer binding. There is also great variability in the methods used to quantify high-affinity receptors in vitro and in vitro (Richfield et al., 1986; De Haes, 2005; Shalgunov, 2017).

The notion of biased agonism introduces more complexity in molecular effects of agonists. A combination of PET and MRI may shed light on agonist functional activities (Vidal et al., 2018). Finally, there is a need to develop new tools to demonstrate in vivo coupling of GPCRs. Indeed, the binding of an agonist radioligand involves intracellular molecular remodeling, which is probably not the case with the binding of a “silent” antagonist. These differences in docking justify that the brain images of agonists, and in particular their distribution patterns, are not directly comparable to those currently obtained by PET imaging of receptors using mainly antagonists. It is therefore necessary to develop new models for interpreting this molecular and functional imaging of receptors. The interpretation of the models will be based in particular on the implementation of PET imaging studies that will compare the binding patterns of an antagonistic radiotracer and an agonist radiotracer directed toward the same receptor in the same subject. Studies on animal models will also provide valuable information, in particular by pharmacologically modulating the G-protein coupling state of receptors during PET acquisitions. Finally, molecular modeling, whose bioinformatics tools are constantly evolving, will make it possible to simulate the docking specificity of agonist molecules in receptor molecular niches.

All these new tools will lead to new paradigms for neuroimaging which, in turn, will contribute to new advances in neurology and psychiatry.

AUTHOR CONTRIBUTIONS

LZ initiated the research topic of the manuscript, proposed its initial plan and made its revision. MC and BV wrote the manuscript.

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SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fnmol.2019.00255/full#supplementary-material

REFERENCES

Addy, C., Wright, H., Van Laere, K., Gantz, I., Erondu, N., Musser, B. J., et al. (2008). The Acyclic CB1R inverse agonist taranabant mediates weight loss by increasing energy expenditure and decreasing caloric intake. Cell Metab. 7, 68–78. doi: 10.1016/j.cmet.2007.11.012

Ahmad, R., Goffin, K., Van den Stock, J., De Winter, F.-L., Cleeren, E., Bormans, G., et al. (2014). In vivo type 1 cannabinoid receptor availability in Alzheimer’s disease. Eur. Neuropsychopharmacol. 24, 242–250. doi: 10.1016/j.europsy.2013.10.002

Albizu, L., Moreno, J. L., Gonzalez-Maeso, J., and Sealfon, S. C. (2010). Heteromerization of G protein-coupled receptors: relevance to neurological disorders and neurotherapeutics. CNS Neurol. Disord. Drug Targets 9, 636–650. doi: 10.2174/187152710793361586

Avisar, S., and Schreiber, G. (2006). The involvement of G proteins and regulators of receptor-G protein coupling in the pathophysiology, diagnosis and treatment of mood disorders. Clin. Chim. Acta 366, 37–47. doi: 10.1016/j.cca.2005.11.003

Aznavour, N., Rhah, I., Riad, M., Reilhac, A., Costes, N., Descarries, L., et al. (2006). A PET imaging study of 5-HT<sub>1A</sub> receptors in cat brain after acute and chronic fluoxetine treatment. Neuroimage 33, 834–842. doi: 10.1016/j.neuroimage.2006.08.012

Battaglia, G., Shannon, M., and Titeler, M. (1984). Guanyl nucleotide and divergent cation regulation of cortical S 2 serotonin receptors. J. Neurochem. 43, 1213–1219. doi: 10.1111/j.1471-4159.1984.tb05375.x

Becker, G., Streichenberger, N., Billard, T., Newman-Tancredi, A., and Zimmer, L. (2014). A postmortem study to compare agonist and antagonist 5-HT<sub>1A</sub> receptor-binding sites in Alzheimer’s disease. CNS Neurosci. Ther. 20, 930–934. doi: 10.1111/cns.12306

Benchicher, B., Fuchs, P., Sheth, R., Dannals, R., Campbell, J., and Frost, J. (2002). Pain activation of human supraspinal opioid pathways as demonstrated by [11C]carfentanil and positron emission tomography (PET). Pain 99, 589–598. doi: 10.1016/s0304-3959(02)00266-x

Benchicher, B., Guarda, A. S., Colantuoni, C., Ravert, H. T., Dannals, R. F., and Frost, J. J. (2005). Regional mu-opioid receptor binding in insular cortex is decreased in bulimia nervosa and correlates inversely with fasting behavior. J. Nucl. Med. 46, 1349–1351.

Benchicher, B., Wand, G. S., McCaul, M. E., Kim, Y. K., Ilgin, N., Dannals, R. F., et al. (2004). Mu-opioid receptor binding measured by [11C]carfentanil positron emission tomography is related to craving and mood in alcohol disorders and neurotherapeutics. CNS Neurol. Disord. Drug Targets 3, 636–650. doi: 10.2174/187152710793361586

Bencherif, B., Fuchs, P., Sheth, R., Dannals, R., Campbell, J., and Frost, J. (2002). Pain activation of human supraspinal opioid pathways as demonstrated by [11C]carfentanil and positron emission tomography (PET). Pain 99, 589–598. doi: 10.1016/s0304-3959(02)00266-x

Benchicher, B., Guarda, A. S., Colantuoni, C., Ravert, H. T., Dannals, R. F., and Frost, J. J. (2005). Regional mu-opioid receptor binding in insular cortex is decreased in bulimia nervosa and correlates inversely with fasting behavior. J. Nucl. Med. 46, 1349–1351.

Benchicher, B., Wand, G. S., McCaul, M. E., Kim, Y. K., Ilgin, N., Dannals, R. F., et al. (2004). Mu-opioid receptor binding measured by [11C]carfentanil positron emission tomography is related to craving and mood in alcohol
dependence. Biol. Psychiatry 55, 255–262. doi: 10.1016/j.biopsych.2003.07.007
Benson, B., Carson, R., Kiesewetter, D., Herscovitch, P., Eckelman, W., Post, R., et al. (2004). A potential cholinergic mechanism of procaine's limbic activation. Neuropsychopharmacology 29, 1239–1250. doi: 10.1038/sj.npp.1304004
Berg, K. A., and Clarke, W. P. (2006). Development of functionally selective agonists as novel therapeutic agents. Drug Discov. Today Ther. Strateg. 3, 421–428. doi: 10.1016/j.ddstr.2006.10.017
Berg, K. A., and Clarke, W. P. (2018). Making sense of pharmacology: inverse agonism and functional selectivity. Int. J. Neuropharmacol. 21, 962–977. doi: 10.1093/ijnp/ppy071
Bjork, K., and Svenningsson, P. (2011). Modulation of monoamine receptors by adaptor proteins and lipid rafts: role in some effects of centrally acting drugs and therapeutic agents. Annu. Rev. Pharmacol. Toxicol. 51, 211–242. doi: 10.1146/annurev-pharm-tox-010510-100520
Boileau, I., Guttman, M., Rusjan, P., Adams, J. R., Houle, S., Tong, J., et al. (2009). Decreased binding of the D3 dopamine receptor-prefering ligand [11C]-(+)-PHNO in drug-naive Parkinson’s disease. Brain 132, 1366–1375. doi: 10.1093/brain/awn337
Braden, M. R., Parrish, J. C., Naylor, J. C., and Nichols, D. E. (2006). Molecular interaction of serotonin 5-HT2A receptor residues Phe339(6.51) and Phe340(6.52) with superpotent N-Benzyl phenethylamine agonists. Mol. Pharmacol. 70, 1956–1964. doi: 10.1124/mol.106.028720
Bruchas, M. R., Yang, T., Schreiber, S., Defino, M., Kwan, S. C., Li, S., et al. (2007). Long-lasting kappa opioid antagonists disrupt receptor signaling and produce noncompetitive effects by activating c-Jun N-terminal kinase. J. Biol. Chem. 282, 29803–29811. doi: 10.1074/jbc.M705540200
Burns, H. D., Van Laere, K., Sanabria-Bohorquez, S., Hamill, T. G., Bornmans, G., Eng, W.-S., et al. (2007). [18F]MK-9470, a positron emission tomography (PET) tracer for in vivo human PET brain imaging of the cannabinoid-1 receptor. Proc. Natl. Acad. Sci. U.S.A. 104, 9800–9805. doi: 10.1073/pnas.0703472104
Campbell, C. M., Bounds, S. C., Kuwabara, H., Edwards, R. R., Campbell, J. N., Haythornthwaite, J. A., et al. (2013). Individual variation in sleep quality and duration is related to cerebral mu opioid receptor binding potential during tonic laboratory pain in healthy subjects. Pain Med. Malden Mass 14, 1882–1892. doi: 10.1111/pme.12331
Cannon, D., Carson, R., Nugent, A., Eckelman, W., Kiesewetter, D., Williams, J., et al. (2006). Reduced muscarinic type 2 receptor binding in subjects with bipolar disorder. Arch. Gen. Psychiatry 63:741. doi: 10.1001/archpsyc.63.7.741
Cannon, D. M., Klaver, J. K., Gandhi, S. K., Solorio, G., Peck, S. A., Erickson, B. R., et al. (2014). Changes in cerebral CB1 receptor availability after acute and chronic alcohol abuse and monitored abstinence. J. Neurosci. Off. J. Soc. Neurosci. 34, 2822–2831. doi: 10.1523/JNEUROSCI.0849-13.2014
Caravaggio, F., Chung, J., Ceccarini, J., Weltens, N., Ly, H. G., Tack, J., Van Oudenhove, L., et al. (2016). Association between cerebral cannabinoid 1 receptor availability and body mass index in patients with food intake disorders and healthy subjects: a [18F]MK-9470 PET study. Transl. Psychiatry 6:e853. doi: 10.1038/tp.2016.118
Ceelen, P., Casteels, C., Bormans, G., Van Laere, K., Janssen, P., et al. (2018). Positron emission tomography imaging of cerebral glucose metabolism and type 1 cannabinoid receptor availability during temporal lobe epileptogenesis in the amygdala kindling model in rhesus monkeys. Epilepsia 59, 959–970. doi: 10.1111/epi.14059
Cohen, R., Carson, R., Filbey, F., Szczepanik, J., and Sunderland, T. (2006). Age and APOE-ε4 genotype influence the effect of phystostigmine infusion on the in vivo distribution volume of the muscarinic-2 receptor dependent tracer [18F]FTZT. Proc. Natl. Acad. Sci. U.S.A. 103, 86–92. doi: 10.1073/pnas.020726
Cohn, R. M., Podruchny, T. A., Bokde, A. L. W., Carson, R. E., Herscovitch, P., Ceccarini, J., Weltens, N., et al. (2013). MK-9470 PET measurement of cannabinoid CB1receptor availability in chronic cannabis users. Addict. Biol. 20, 357–367. doi: 10.1111/adb.12116
Colom, R., Costes, C., Goffin, K., Koole, M., Van Laere, K., and glucose metabolism. Eur. J. Nucl. Med. Mol. Imaging 37, 2354–2363. doi: 10.1007/s00259-010-1574-2
Casteels, C., Vanbilloen, B., Vercammen, D., Bosier, B., Lambert, D. M., Bormans, G., et al. (2018d). Influence of chronic bromocriptine and levodopa administration on cerebral type 1 cannabinoid receptor binding. Synapse 64, 617–623. doi: 10.1002/syn.22907
Casteels, C., Gérard, N., van Kuyck, K., Pottel, L., Nuttin, B., Bormans, G., et al. (2014). Small animal PET imaging of the type 1 cannabinoid receptor in a rodent model for anorexia nervosa. Eur. J. Nucl. Med. Mol. Imaging 41, 308–321. doi: 10.1007/s00259-013-2522-8
Casteels, C., Koole, M., Celen, S., Bormans, G., and Van Laere, K. (2012). Preclinical evaluation and quantification of [18F]MK-9470 as a radioligand for PET imaging of the type 1 cannabinoid receptor in rat brain. Eur. J. Nucl. Med. Mol. Imaging 39, 1467–1477. doi: 10.1007/s00259-012-2163-3
Casteels, C., Vanpeperstraete, C., Rangarajan, J. R., Dresselaers, T., Riess, O., Bormans, G., et al. (2011). Metabolic and type 1 cannabinoid receptor imaging of a transgenic rat model in the early phase of Huntington disease. Exp. Neurol. 229, 440–449. doi: 10.1016/j.expneurol.2011.03.014
Ceccarini, J., Ahmad, R., Van de Vliet, L., Casteels, C., Vandenbulcke, M., Vandenberghe, W., et al. (2019). Behavioral symptoms in premanifest huntington disease correlate with reduced frontal CB 1 R levels. J. Nucl. Med. 60, 115–121. doi: 10.2967/jnumed.118.210393
Elsinga, P., Kawamura, K., Senda, M., Vaalburg, W., De Lean, A., De Haes, U. (2005). Synthesis and evaluation of [11C]MNPA in cynomolgus monkey. Nucl. Med. Biol. 32, 535ñ540. doi: 10.1016/j.nucmedbio.2005.01.007

Finnema, S. J., Stepanov, V., Ettrup, A., Nakao, R., Amini, N., Svedberg, M., et al. (2014). Characterization of [11C]Cimbi-36 as an agonist PET radioligand for the 5-HT2A and 5-HT2C receptors in the nonhuman primate brain. Neuroimage 84, 342ñ353. doi: 10.1016/j.neuroimage.2013.08.035

Frost, J., Mayberg, H., Svarer, C., McMahon, B., da Cunha-Bang, S., Lehel, S., Møller, K., et al. (2012). Reduced basal ganglia serotonin type 1A (5-HT1A) receptor occupancy in patients with chronic tension-type headache. Ann. Neurol. 72, 873ñ883. doi: 10.1002/ana.23587

Frost, J., Mayberg, H., Fisher, R., Douglass, K., Dannals, R., Links, J., et al. (1989). Multicompartmental analysis of [11C]Carfentanil binding to opiate receptors in human brains measured by positron emission tomography. J. Cereb. Blood Flow Metab. 9, 398ñ409. doi: 10.1016/j.clinph.1989.59

Frost, J., Mayberg, H., Sadzot, B., Dannals, R., Lever, J., Ravert, H., et al. (1990). Comparison of [11C]Diprenorphine and [11C]Carfentanil binding to opiate receptors in human brains by positron emission tomography. J. Cereb. Blood Flow Metab. 10, 484ñ492. doi: 10.1038/jcbfm.1990.90

Frost, J., Wagner, H., Dannals, R., Ravert, H., Links, J., Wilson, A., et al. (1989). Imaging opiate receptors in the human brain by positron tomography. J. Comput. Assist. Tomogr. 13, 231ñ237. doi: 10.1097/00004728-198503000-00001

Frost, J., Mayberg, H., Kula, N., and Neumeyer, J. (1990). Synthesis and evaluation of a series of substituted 11C-nicotine acetylcholine receptor ligands. J. Med. Chem. 33, 657ñ663. doi: 10.1021/jm00168a040

Frost, J., Wagner, H., Dannals, R., Kula, N., and Neumeyer, J. (1990). Synthesis and evaluation of 11C-labeled (S)-N-[(2-phenylethyl)pyrrolidin-2-yl]methyl-3-methylthiobenzamide as a PET 5-HT1A receptor ligand. Nucl. Med. Biol. 27, 567ñ573. doi: 10.1016/s0969-8051(02)00305-0

Gallezot, J., Esterlis, I., Bois, F., Zheng, M., Lin, S., Kloczynski, T., et al. (2014). Evaluation of the sensitivity of the novel 4\(\alpha\)2 nicotinic acetylcholine receptor PET radioligand 18F (\(\alpha\)4\(\alpha\)2-NCHEB to increases in synaptic acetylcholine levels in rhesus monkeys. Synapse 68, 56ñ56. doi: 10.1002/syn.21767

Gao, M., Wang, M., and Zheng, Q. (2012). A new high-yield synthetic route to PET CB1 radioligands [11C]OMAR and its analogs. Bioorg. Med. Chem. Lett. 22, 3704ñ3709. doi: 10.1016/j.bmcl.2012.04.030

Gao, Y., Baldessarini, R., Kula, N., and Neumeyer, J. (1990). Synthesis and dopamine receptor affinity of enantiomers of 2-substituted apomorphines and their N-n-propyl analogs. J. Med. Chem. 33, 1800ñ1805. doi: 10.1021/jm00168a040

Gérard, N., Ceccarini, J., Bormans, G., and Van Laere, K. (2011). Brain nigrostriatal dopamine type 1A (DA1) receptors and their role in Parkinson’s disease. Curr. Top. Med. Chem. 11, 232, 4129ñ4157. doi: 10.2174/15675241013079317837

Gérard, N., Pieters, G., Goffin, K., Bormans, G., and Van Laere, K. (2011). Brain dopaminergic type 1 cannabinoid receptor availability in patients with anorexia and bulimia nervosa. Biol. Psychiatry 70, 777ñ784. doi: 10.1016/j.biopsych.2011.05.010

Ghita, U. E., Preston, K. L., Epstein, D. H., Kuwabara, H., Endres, C. J., Bencherif, B., et al. (2010). Brain mu-opioid receptor binding predicts treatment outcome in cocaine-abusing outpatients. Biol. Psychiatry 68, 697ñ703. doi: 10.1016/j.biopsych.2010.05.003

Girotini, V. (2005). Imaging the dopamine system with in vivo [11C]raclopride displacement studies: understanding the true mechanism. Mol. Imaging Biol. Off. Publ. Acad. Mol. Imaging 7, 4ñ52. doi: 10.1007/s11305-005-0952-0

Girotini, V., Galineau, L., Willett, M., Mizrahi, R., Bloomfield, P., Seeman, P., et al. (2004a). Binding characteristics and sensitivity to endogenous dopamine of [11C](+)-PHNO, a new agonist radiotracer for imaging the high-affinity state of D2 receptors in vivo using positron emission tomography. J. Neurochem. 97, 1089ñ1103. doi: 10.1111/j.1471-4159.2006.03840.x
Ginovart, N., Willett, M., Rusjan, P., Graff, A., Bloomfield, P., Houle, S., et al. (2006b). Positron emission tomography quantification of [11C](-)-PHNO binding in the human brain. J. Cereb. Blood Flow Metab. 27, 857–871. doi: 10.1038/sj.jcbf.9600411

Goffin, K., Bormans, G., Castréels, C., Bosier, B., Lambert, D., Grachev, L., et al. (2008). An in vivo [18F] MK-9470 microPET study of type 1 cannabinoid receptor binding in Wistar rats after chronic administration of valproate and levetiracetam. Neuropharmacology 54, 1103–1106. doi: 10.1016/j.neuropharm.2008.02.018

Goffin, K., Van Paesschen, W., and Van Laere, K. (2011). In vivo agonists and inverse agonists in PET Neuroimaging. In vivo 25, 91–102. doi: 10.2967/jin.110.071758

Graff-Guerrero, A., Mizrahi, R., Agid, O., Marcon, H., Barsoum, P., Rusjan, P., et al. (2007). Buprenorphine duration of action: mu-opioid receptor availability predicts cold pressor pain threshold in healthy human subjects. J. Neurosci. 27, 857–871. doi: 10.1523/jneurosci.3676-06.2007

Hajós, L., Darvas, G., Horváth, T., Hruska, K., Fodor, F., and Nemeth, G. (2003). High occupancy of σ1 receptors in the striatum of the rat. Eur. J. Pharmacol. 461, 125–131. doi: 10.1016/s00142999(03)02576-5

Härmä, M., Hirth, N., Remold, M., Batra, A., Smolka, M., Hoffmann, S., et al. (2017). Low μ-opioid receptor status in alcohol dependence identified by combined positron emission tomography and post-mortem brain analysis. Neuropharmacology. Off. Publ. Am. Coll. Neuropsychopharmacol. 42, 606–614. doi: 10.1038/npp.2016.145

Hoffman, B. B., and Lefkowitz, R. J. (1980). Radioligand binding studies of adrenergic receptors: new insights into molecular and physiological regulation. Annu. Rev. Pharmacol. Toxicol. 20, 581–608. doi: 10.1146/annurev.pa.20.040180.003053

Honer, M., Gobbi, L., Martarello, L., and Comley, R. A. (2014). Radioligand development of molecular imaging of the central nervous system with positron emission tomography. Drug Discov. Today 19, 1936–1944. doi: 10.1016/j.drudis.2014.08.012

Horst, A. S., Attwood, M. M., Rask-Andersen, M., Schiöth, H. B., and Harris, R. E., Zubieta, J.-K., Scott, D. J., Napadow, V., Gracely, R. H., and Clauw, D. J. (2009). Traditional Chinese acupuncture and placebo (sham) acupuncture are differentiated by their effects on mu-opioid receptors (MORs). Psychopharmacology 108, 16–22. doi: 10.1007/bf02245279

Horst, A. S., Attwood, M. M., Rask-Andersen, M., Schiöth, H. B., and Harris, R. E., Zubieta, J.-K., Scott, D. J., Napadow, V., Gracely, R. H., and Clauw, D. J. (2009). Traditional Chinese acupuncture and placebo (sham) acupuncture are differentiated by their effects on mu-opioid receptors (MORs). Psychopharmacology 108, 16–22. doi: 10.1007/bf02245279

Huang, D., Narendran, R., Hwang, Y., Slifstein, M., Talbot, P., Sudo, Y., et al. (2004). Quantitative analysis of (1)N-11C-Propyl-Norapomorphine in vivo binding in nonhumanprimates. J. Nucl. Med. 45, 338–346.

Hwang, D., Narendran, R., and Laruelle, M. (2005). Positron-labeled dopamine agonists for probing the high affinity states of dopamine subtype 2 receptors. Bioconjugate Chem. 16, 27–31. doi: 10.1021/bc0409834

Hwang, D. R., Kegeles, L. S., and Laruelle, M. (2000). (-)-N-[11C]propyl-norapomorphine: a positron-labeled dopamine agonist for PET imaging of D2 receptors. Nucl. Med. Biol. 27, 533–539. doi: 10.1016/s0960-8550(01)00144-x

Ingman, K., Hagberg, N., Aalto, S., Någren, K., Juhasoksi, A., Karhuvaara, S., et al. (2009). Low opioid receptor status in alcohol dependence identified by combined positron emission tomography and post-mortem brain analysis. Neuropharmacology. Off. Publ. Am. Coll. Neuropsychopharmacol. 42, 606–614. doi: 10.1038/npp.2016.145

Hoffman, B. B., and Lefkowitz, R. J. (1980). Radioligand binding studies of adrenergic receptors: new insights into molecular and physiological regulation. Annu. Rev. Pharmacol. Toxicol. 20, 581–608. doi: 10.1146/annurev.pa.20.040180.003053

Honer, M., Gobbi, L., Martarello, L., and Comley, R. A. (2014). Radioligand development of molecular imaging of the central nervous system with positron emission tomography. Drug Discov. Today 19, 1936–1944. doi: 10.1016/j.drudis.2014.08.012

Horti, A. (2007). “[11C]JHU75528, A PET radioligand for imaging of cerebral cannabinoid CB1 receptors,” in Proceedings of the 39-th Meeting of European Brain and Behaviour Society, Trieste.

Horti, A. G., Fan, H., Kuwabara, H., Hilton, J., Ravert, H. T., Holt, D. P., et al. (2006). [11C]JHU75528: a radiotracer for PET imaging of CB1 cannabinoid receptors. J. Nucl. Med. Off. Publ. Soc. Nucl. Med. 47, 1689–1696.

Hus, D. T., Sanford, B. J., Meyers, K. K., Love, T. M., Hazlett, K. E., Walker, S. J., et al. (2015). It still hurts: altered endogenous opioid activity in the brain during social rejection and acceptance in major depressive disorder. Mol. Psychiatry 20, 193–200. doi: 10.1038/mp.2014.185

Hus, D. T., Sanford, B. J., Meyers, K. K., Love, T. M., Hazlett, K. E., Wang, H., et al. (2013). Response of the μ-opioid system to social rejection and acceptance. Mol. Psychiatry 18, 1211–1217. doi: 10.1038/mp.2013.96

Hwang, D., Narendran, R., Hwang, Y., Slifstein, M., Talbot, P., Sudo, Y., et al. (2004). Quantitative analysis of (1)N-11C-Propyl-Norapomorphine in vivo binding in nonhumanprimates. J. Nucl. Med. 45, 338–346.

Hwang, D., Narendran, R., and Laruelle, M. (2005). Positron-labeled dopamine agonists for probing the high affinity states of dopamine subtype 2 receptors. Bioconjugate Chem. 16, 27–31. doi: 10.1021/bc0409834

Hwang, D. R., Kegeles, L. S., and Laruelle, M. (2000). (-)-N-[11C]propyl-norapomorphine: a positron-labeled dopamine agonist for PET imaging of D2 receptors. Nucl. Med. Biol. 27, 533–539. doi: 10.1016/s0960-8550(01)00144-x

Ingman, K., Hagberg, N., Aalto, S., Någren, K., Juhasoksi, A., Karhuvaara, S., et al. (2009). Prolonged central mu-opioid receptor occupancy after single and repeated nalmefene dosing. Neuropharmacology. Off. Publ. Am. Coll. Neuropsychopharmacol. 30, 2245–2253. doi: 10.1016/s0028-3908(03)00367-x
Johansen, A., Hansen, H. D., Svarer, C., Lehel, S., Leth-Petersen, S., Kristensen, J.-C., Colom et al. Agonists in PET Neuroimaging

Jitsuoka, M., Tsukahara, D., Ito, S., Tanaka, T., Takenaga, N., Tokita, S., et al.

Karlsson, H. K., Tuulari, J. J., Tuominen, L., Hirvonen, J., Honka, H., Parkkola, R., Helin, I., Ishiwata, K., Oda, K., Sakata, M., Kimura, Y., Kawamura, K., et al.

Jastrzebska, B., Debinski, A., Filipek, S., and Palczewski, K. (2011). Role of dopamine D2 receptor availability in the brain. J. Cereb. Blood Flow Metab. 31, 1607–1613. doi: 10.1038/jcbfm.2010.16

Johansen, A., Hansen, H. D., Svarer, C., Lehel, S., Leth-Petersen, S., Kristensen, J.-L., et al. (2018). The importance of small polar radiomolecules in molecular neuroimaging: a PET study with [11C]CimbI-36 labeled in two positions. J. Cereb. Blood Flow Metab. Off. J. Int. Soc. Cereb. Blood Flow Metab. 38, 659–668. doi: 10.1002/jbfm.21746

Jones, J. H., Anderson, P. S., Baldwin, J. J., Clineschmidt, B. V., McClure, D. E., et al. (1984). Synthesis of 4-substituted 2H-naphth[1,2-b]-1,4-oxazines, a new class of dopamine agonists. J. Med. Chem. 27, 1607–1613. doi: 10.1021/jm00378a014

Jorgensen, L. M., Weikop, P., Villadsen, J., Visnapuu, T., Etrup, A., Hansen, H. D., et al. (2017). Cerebral 5-HT receptor expression correlates with [11C]CimbI-36 PET measures of 5-HT2A receptor occupancy in the pig brain. J. Cereb. Blood Flow Metab. 37, 425–434. doi: 10.1002/jbfm.21768

Joutsjoki, A., Karlsson, H. K., Majuri, J., Nuutila, P., Helin, S., Kaasinen, V., et al. (2001). Binge eating disorder and morbid obesity are associated with lowered mu-opioid receptor availability in the brain. Psychiatry Res. Neuroimaging 120, 254–257. doi: 10.1016/s0925-5773(01)00184-5

Kasprzak, I., Kuwabara, H., Heishman, S. J., Brasic, J. R., Contoreggi, C., Cascella, N., Mackowick, K. M., et al. (2014). Mu opioid receptor binding correlates with nicotine dependence and reward in smokers. PLoS One 9:e113694. doi: 10.1371/journal.pone.0113694

Kawamura, K., Svarer, C., Lehel, S., Leth-Petersen, S., Kristensen, J.-C., Colom et al. Agonists in PET Neuroimaging

Kawamura, K., Tsukada, H., Kawamura, K., Ninomiya, Y., Sato, K., Lin, J.-H., et al. (2007). Synthesis and evaluation of fluorine-18-labeled SA4503 as a selective sigma1 receptor ligand for positron emission tomography. Molec. Imaging. 34, 571–577. doi: 10.1016/j.mrcibo.2007.03.009

Keith, D. E., Anton, B., Murray, S. R., Zaki, P. A., Chu, P. C., Lissin, D. V., et al. (1998). mu-Opioid receptor internalization: opiate drugs have differential effects on a conserved endocytotic mechanism in vitro and in the mammalian brain. Mol. Pharmacol. 53, 377–384. doi: 10.1124/mol.53.3.377

Kenakin, T. (2015). New lives for seven transmembrane receptors as drug targets. Trends Pharmacol. Sci. 36, 705–706. doi: 10.1016/j.tips.2015.09.004

Kenakin, T., and Christopoulos, A. (2012). Signalling bias in new drug discovery: detection, quantification and therapeutic impact. Nat. Rev. Drug Discov. 12, 205–216. doi: 10.1038/nrd3954

Kubota, M., Nagashima, T., et al. (2003b). An increase of sigma1 receptors in the aged monkey brain. Neurobiol. Aging 24, 745–752. doi: 10.1016/s0197-4580(02)00152-5

Kumar, J. S. D., Maiti, M. S., Majo, V. J., Prabhakaran, J., Mali, P., Savenkova, L., et al. (2012). Comparison of high and low affinity serotonin 1A receptors by PET in vivo in nonhuman primates. J. Pharmacol. Sci. 120, 254–257. doi: 10.1254/jphs.121005C

Kwabara, H., Ishiwata, K., Tajima, H., Shiba, K., Tsuji, C., Harada, N., and Kusaka, M. (2000). In vivo evaluation of [11C]SA4503 as a PET ligand for mapping CNS sigma1 receptors. Nucl. Med. Biol. 27, 235–261. doi: 10.1016/s0969-8051(00)0081-0

Kawamura, K., Ishiwata, K., Tajima, H., Shiba, K., et al. (2003a). Synthesis and evaluation of [11C]SA4503 as a PET ligand for mapping CNS sigma1 receptors. Nucl. Med. Biol. 30, 273–284. doi: 10.1016/s0969-8051(02)00439-0

Kawamura, K., Kumar, J., Tsukada, H., Shibata, Y., Kobayashi, T., Nishiyama, S., Kakuichi, T., et al. (2003b). An increase of sigma1 receptors in the aged monkey brain. Neurobiol. Aging 24, 745–752. doi: 10.1016/s0197-4580(02)00152-5

Kawamura, K., Ishiwata, K., Tajima, H., Shiba, K., et al. (2000). In vivo evaluation of [11C]SA4503 as a PET ligand for mapping CNS sigma1 receptors. Nucl. Med. Biol. 27, 235–261. doi: 10.1016/s0969-8051(00)0081-0

Kawamura, K., Ishiwata, K., Tajima, H., Shiba, K., et al. (2003a). Synthesis and evaluation of [11C]SA4503 as a PET ligand for mapping CNS sigma1 receptors. Nucl. Med. Biol. 30, 273–284. doi: 10.1016/s0969-8051(02)00439-0

Kawamura, K., Kumar, J., Tsukada, H., Shibata, Y., Kobayashi, T., et al. (2003b). An increase of sigma1 receptors in the aged monkey brain. Neurobiol. Aging 24, 745–752. doi: 10.1016/s0197-4580(02)00152-5

Kawamura, K., Ishiwata, K., Tajima, H., Shiba, K., et al. (2000). In vivo evaluation of [11C]SA4503 as a PET ligand for mapping CNS sigma1 receptors. Nucl. Med. Biol. 27, 235–261. doi: 10.1016/s0969-8051(00)0081-0

Kawamura, K., Ishiwata, K., Tajima, H., Shiba, K., et al. (2003a). Synthesis and evaluation of [11C]SA4503 as a PET ligand for mapping CNS sigma1 receptors. Nucl. Med. Biol. 30, 273–284. doi: 10.1016/s0969-8051(02)00439-0

Kawamura, K., Kumar, J., Tsukada, H., Shibata, Y., Kobayashi, T., et al. (2003b). An increase of sigma1 receptors in the aged monkey brain. Neurobiol. Aging 24, 745–752. doi: 10.1016/s0197-4580(02)00152-5

Kawamura, K., Ishiwata, K., Tajima, H., Shiba, K., et al. (2000). In vivo evaluation of [11C]SA4503 as a PET ligand for mapping CNS sigma1 receptors. Nucl. Med. Biol. 27, 235–261. doi: 10.1016/s0969-8051(00)0081-0

Kawamura, K., Ishiwata, K., Tajima, H., Shiba, K., et al. (2003a). Synthesis and evaluation of [11C]SA4503 as a PET ligand for mapping CNS sigma1 receptors. Nucl. Med. Biol. 30, 273–284. doi: 10.1016/s0969-8051(02)00439-0
la Cour, C. M. (2006). Regional differences in the coupling of 5-
Hydroxytryptamine-1A receptors to G proteins in the rat brain. Mol.
Pharmacol. 70, 1013–1021. doi: 10.1124/mol.106.027756

Laruelle, M. (2000). Imaging synaptic neurotransmission with in vivo binding
competition techniques: a critical review. J. Cereb. Blood Flow Metab.
20, 427–451. doi: 10.1097/00006477-200003000-00001

Luttrell, L. M., Maudsley, S., and Bohn, L. M. (2015). Fulfilling the promise of
Biased G protein-coupled receptor agonism.

Light, S. N., Bieliauskas, L. A., and Zubieta, J.-K. (2017). Top-Down mu-opioid
receptor function in humans: mu-opioid receptors in ventrolateral prefrontal
cortex mediate the relationship between hedonic tone and executive function
in major depressive disorder. J. Neuropsychiatry Clin. Neurosci. 29, 357–364.
doi: 10.1176/appi.neuropsych.16090171

Manninen, S., Tuominen, L., Dunbar, R. I., Karjalainen, T., Hirvonen, J., Arponen,
E., et al. (2017). Social laughter triggers endogenous opioid release in humans.
J. Neurosci. Off. J. Soc. Neurosci. 37, 6125–6131. doi: 10.1523/JNEUROSCI.
0688-16-2017

Matsunawa, I. K., Peciña, M., Love, T. M., Nuechterlein, E. B., Cummingford,
C. M., Green, C. R., et al. (2013). Alterations in endogenous opioid functional
measures in chronic back pain. J. Neurosci. Off. J. Soc. Neurosci. 33, 14729–
14737. doi: 10.1523/JNEUROSCI.1400-13.2013

Mathis, C. A., Huang, Y., and Simpson, N. R. (1997). Synthesis and evaluation of 5-
HT1A agonists a radioligands: failure of G protein-coupled receptor agonists as
in vivo imaging agents. J. Label Compd. Radiopharm. 40, 563–564.

Matsusue, D., Gaiser, E., Gallezot, J., Angarita, G., Pittman, B., Nabulsi, N., et al.
(2015). A preliminary study of dopamine D2/3 receptor availability and social
status in healthy and cocaine dependent humans imaged with [11C](+)-PHNO.
Drug Alcohol Depend. 154, 167–173. doi: 10.1016/j.drugalcdep.2015.06.039

Mayberg, H., Sadzot, B., Meltzer, C., Fisher, R., Lesser, D., Dannals, R., et al.
(1991). Quantification of mu and non-mu opioid receptors in temporal lobe epilepsy
using positron emission tomography. Ann. Neurol. 30, 3–11. doi: 10.1002/ana.
410300103

McCormick, P., Ginovart, N., and Wilson, A. (2010). Isofuran anaesthesia
differentially affects the amphetamine sensitivity of agonist and antagonist
D2/D3 positron emission tomography radiotracers: implications for in vivo imaging
of dopamine release. Mol. Imaging Biol. 13, 737–746. doi: 10.1007/s10045-
010-0380-3

McCormick, P. N., Kapur, S., Reckless, G., and Wilson, A. A. (2009). Ex vivo [11
Cl]-(+)-PHNO binding is unchanged in animal models displaying increased
high-affinity states of the D 2 receptor in vitro. Synapse 63, 998–1009. doi:
10.1002/syn.20671

McCormick, P. N., Kapur, S., Seeman, P., and Wilson, A. A. (2008). Dopamine D2 receptor radiotracers [11C](+)-PHNO and [3H] raclopride are
indistinguishably inhibited by D2 agonists and antagonists ex vivo. Nucl.
Med. Biol. 35, 11–17. doi: 10.1016/j.nucmedbio.2007.08.005

Mick, I., Myers, J., Ramos, A. C., Stokes, P. R. A., Erritzoe, D., Colasanti, A.,
et al. (2016). Blunted endogenous opioid release following an oral amphetamine
challenge in pathological gamblers. Neuropsychopharmacol. Off. Publ. Am. Coll.
Nucl. Med. Pharmacol. 41, 1742–1750. doi: 10.1038/np.2015.340

Miederer, L., Buchholz, H.-G., Kronfeld, A., Maus, S., Weyer-Elbicher, V.,
Mildenberger, P., et al. (2018). Pharmacokinetics of the cannabinoid receptor
ligand [18 F]MK-9470 in the rat brain - Evaluation of models using microPET.
Med. Phys. 45, 725–734. doi: 10.1002/mp.12732

Milak, M. S., Severance, A. J., Prabhakaran, J., Kumar, J. D., Majo, V. I.,
Ogden, R. T., et al. (2011). In vivo serotonin-sensitive binding of [11 C]CUMI-101:
a serotonin 1A receptor agonist positron emission tomography radiotracers.
J. Cereb. Blood Flow Metab. 31, 243–249. doi: 10.1038/jcbfm.2010.83

Miller, J. M., Zanderigo, F., Purushothaman, P. D., Delorenzo, C., Rubin-Falcone,
H., Ogden, R. T., et al. (2018). kappa opioid receptor binding in major depression:
a pilot study. Synapse 72:e22042. doi: 10.1002/syn.22042

Minkowski, C. P., Epstein, D., Frost, J. J., and Gorelick, D. A. (2012). Differential
response to IV carfentanil in chronic cocaine users and healthy controls. Addict.
Biol. 17, 149–155. doi: 10.1111/j.1369-1600.2010.00256.x

Minuzzi, L., and Cumming, P. (2010). Agonist binding fraction of dopamine D2/3
receptors in rat brain: a quantitative autodagographic study. Neurochem.
Int. 56, 747–752. doi: 10.1016/j.neuint.2010.01.010

Mishina, M., Ishiwata, K., Ishii, K., Kitamura, S., Kimura, Y., Kawamura, K., et al.
(2005). Function of sigma1 receptors in Parkinson’s disease. Acta Neurol.
Scand. 112, 103–107. doi: 10.1111/j.1600-0404.2005.00432.x

Mitchell, J. M., O’Neill, J. P., Jagust, W. J., and Fields, H. L. (2013). Catechol-O-
methyltransferase genotype modulates opioid release in decision circuitry.
Clim. Transl. Sci. 6, 400–403. doi: 10.1111/cts.12075

Mitchell, J. M., O’Neill, J. P., Janabi, M., Marks, S. M., Jagust, W. J., and Fields,
H. L. (2012). Alcohol consumption induces endogenous opioid release in the
human orbitofrontal cortex and nucleus accumbens. Sci. Transl. Med. 4, 114ra6.
doi: 10.1126/scitranslmed.3002902

Mizrahi, R., Agid, O., Borlido, C., Suridjan, I., Rusjan, P., Houle, S., et al.
(2011). Effects of antipsychotics on D3 receptors: a clinical PET study in
first episode antipsychotic-naive patients with schizophrenia using [11C](-)-N-n-propylnorapomorphine. Schizophr. Res. 131, 63–68. doi: 10.1016/j.schres.2011.05.005

Mizrahi, A., Suris, J., Kenk, M., George, T., Wilson, A., Houle, S., et al. (2012). Dopamine response to psychosocial stress in chronic cannabis users: A PET study with [11C]-PHNO. Neuropsychopharmacology 37, 673–682. doi: 10.1038/npp.2012.232

Mongeau, R., Welner, S. A., Quirion, R., and Suranyi-Cadotte, R. E. (1992). Further evidence for differential affinity states of the serotonin1A receptor in rat hippocampus. Brain Res. 590, 229–238. doi: 10.1016/0006-8993(92)91100-s

Naganawa, M., Jacobsen, L. K., Zheng, M.-Q., Lin, S.-F., Banerjee, A., Byon, W., et al. (2014). Evaluation of the agonist PET radioligand [11C]GR105454 to image kappa opioid receptor in humans: kinetic model selection, test-retest reproducibility and receptor occupancy by the antagonist PF-04455242. Neuroimage 99, 69–79. doi: 10.1016/j.neuroimage.2014.05.033

Naredran, R. (2005). Measurement of the proportion of D2 receptors configured in state of high affinity for agonists in vivo: a positron emission tomography study using [11C]N-Propyl-norapomorphine and [11C]Raclopride in Baboons. J. Pharmacol. Exp. Ther. 315, 80–90. doi: 10.1124/jpet.105.090968

Naredran, R., Frankle, W., Mason, N., Laymon, C., Lopresti, B., Price, J., et al. (2009). Positron emission tomography imaging of D2/3 agonist binding in healthy human subjects with the radiotracer. Synapse 63, 574–584. doi: 10.1002/syn.20633

Naredran, R., Hwang, D., Slièfstein, M., Talbot, P., Erritzoe, D., Huang, Y., et al. (2004). In vivo vulnerability to competition by endogenous dopamine: comparison of the D2 receptor agonist radiotracer (-)-N-[11C]Propyl-norapomorphine ([11C]NPA) with the D2 receptor antagonist radiotracer [11C]Raclopride. Synapse 52, 188–208. doi: 10.1002/syn.20013

Naredran, R., Martinez, D., Mason, N. S., Lopresti, B. J., Himes, M. L., Chen, C.-M., et al. (2011). Imaging of dopamine D2/3 agonist binding in cocaine dependence: a [11C]NPA positron emission tomography study. Synapse 65, 1344–1349. doi: 10.1002/syn.20970

Naredran, R., Mason, N., Laymon, C., Lopresti, B., Velasquez, N., May, M., et al. (2010). A comparative evaluation of the dopamine D2/3 agonist radiotracer [11C]-N-Propyl-norapomorphine and Antagonist [11C]Raclopride to measure amphetamine-induced dopamine release in the human striatum. J. Pharmacol. Exp. Ther. 333, 533–539. doi: 10.1124/pt.109.163501

Naredran, R., Slièfstein, M., Hwang, D., Hwang, Y., Slièfstein, M., et al. (2006). Amphetamine-induced dopamine release: duration of action as assessed with the D2/3 receptor agonist radiotracer (-)-N-[11C]propyl-norapomorphine ([11C]NPA) in an anesthetized nonhuman primate. Synapse 61, 106–109. doi: 10.1002/syn.20346

Neumeyer, J., Neustadt, B., Oh, K., Weinhardt, K., Boyce, C., Rosenberg, F., et al. (1973). Aporphines. 8. synthesis and pharmacological evaluation of (-)-Aphorminol, (±)-Aphocinol, (±)-N-Propyl-norapomorphine, and (±)-N-n-Propyl-norapocodeine. J. Med. Chem. 16, 1223–1228. doi: 10.1021/jm0029401

Newman-Tancredi, A. (2011). Biased agonism at serotonin 5-HT1A receptors – opioid neurotransmitter function in major depression and vulnerability to amphetamine-evoked dopamine release in rat. Neurochem. Int. 58, 243–256. doi: 10.1016/j.neuint.2010.12.007

Paton, C. P., Bock, W. E., Briner, A. K., De Jong, J. D. A., and Waisman, G. M. (2010). Measuring endogenous 5-HT release by emission tomography: promises and pitfalls. J. Cereb. Blood Flow Metab. 30, 1682–1706. doi: 10.1038/jcbfm.2010.104

Peciña, M., Bohnert, A. S. B., Sikora, M., Avery, E. T., Langenecker, S. A., Mickey, B. J., et al. (2015a). Association between placebo-activated neural systems and antidepressant response: neurochemistry of placebo effects in major depression. JAMA Psychiatry 72,1087. doi: 10.1001/jamapsychiatry.2015.1335

Peciña, M., Love, T., Stohler, C. S., Goldman, D., and Zubeta, J.-K. (2015b). Effects of the Mu opioid receptor polymorphism (OPRM1 A118G) on pain regulation, placebo effects and associated personality trait measures. Neuropsychopharmacol. Off. Publ. Am. Coll. Neuropsychopharmacol. 40, 957–965. doi: 10.1038/npp.2014.272

Pejchal, T., Foley, M. A., Kosofsky, B. E., and Waeger, C. (2002). Chronic fluoxetine treatment selectively uncouples raphé 5-HT1A receptors as measured by [35S]-GTPγS autoradiography. Br. J. Pharmacol. 135, 1115–1122. doi: 10.1038/j/sj.1002755.

Peng, T., Zysk, J., Dorff, P., Eilmore, C. S., Ström, P., Malmquist, J., et al. (2010). D2 receptor occupancy in conscious rat brain is not significantly distinguished with [3H]-MNPA, [3H]-(–)-PHNO, and [3H]-raclopride. Synapse 64, 624–633. doi: 10.1002/syn.20771

Pike, V. W. (2009). PET radiotracers: crossing the blood–brain barrier and surviving metabolism. Trends Pharmacol. Sci. 30, 431–440. doi: 10.1016/j.tips.2009.05.005

Placzek, M. S., Schroeder, F. A., Che, T., Wey, H.-Y., Neelamegam, R., Wang, C., et al. (2018). Discrepancies in kappa opioid agonist binding revealed through PET imaging. ACS Chem. Neurosci. 10, 384–395. doi: 10.1021/acschemneuro.8b00293

Placzek, M. S., Van de Bittner, G. C., Wey, H.-Y., Lukas, S. E., and Hooker, J. M. (2015). Immediate and persistent effects of salvinorin A on the kappa opioid receptor in rodents, monitored in vivo with PET. Neuropsychopharmacol. Off. Publ. Am. Coll. Neuropsychopharmacol. 40, 2865–2872. doi: 10.1038/npp.2015.159

Podoruchny, T. A., Connolly, C., Bokde, A., Herscovitch, P., Eckelman, W. C., Kiesewetter, D. O., et al. (2003). In vivo muscarinic 2 receptor imaging in cognitively normal young and older volunteers. Synapse 48, 39–44. doi: 10.1002/syn.10165

Prante, O., Tietjen, R., Rangarajan, J. R., Vuncikx, K., Valdeolivas, S., Maes, F., et al. (2014). Early decrease of type 1 cannabinoid receptor binding and phosphodiesterase 10A activity in vivo in R6/2 Huntingdon mice. Neurobiol. Aging 35, 2858–2869. doi: 10.1016/j.neurobiolaging.2014.06.010

Otsuka, T., Ito, H., Halldin, C., Takahashi, H., Takanoh, H., Arakawa, R., et al. (2009). Quantitative PET analysis of the dopamine D2 receptor agonist radioligand 11C-R-CHSO-N-n-Propynorapomorphine in the human brain. J. Nucl. Med. 50, 703–710. doi: 10.2967/jnumed.108.058503

Palmer, M., Kjaerby, C., Knudsen, G., and Cumming, P. (2011). Effects of unilateral 6-OHDA lesions on [3H]-n-propynorapomorphine binding in striatum ex vivo and vulnerability to amphetamine-evoked dopamine release in rat. Neurochem. Int. 58, 243–256. doi: 10.1016/j.neuint.2010.12.007

Paterson, L. M., Tyacke, R. J., Nutt, D. J., and Knudsen, G. M. (2010). Measuring endogenous 5-HT release by emission tomography: promises and pitfalls. J. Cereb. Blood Flow Metab. 30, 1682–1706. doi: 10.1038/jcbfm.2010.104
Quelch, D., De Santis, V., Streege, A., Myers, J., Wells, L., Nutt, D., et al. (2014). Influence of agonist induced internalization on [3H]Ro15-4513 binding-an application to imaging fluctuations in endogenous GABA with positron emission tomography. Synapse 69, 60–65. doi: 10.1002/syn.21780

Rabiner, E. A., Beaver, J., Malowana, A., Searle, G., Long, C., Nathan, P. J., et al. (2011). Pharmacological differentiation of opioid receptor antagonists by molecular and functional imaging of target occupancy and food rewarded-related brain activation in humans. Mol. Psychiatry 16,785. doi: 10.1038/mp.2011.29

Ramakrishnan, N., Visser, A., Rybczynska, A., Nyakas, C., Luiten, P., Kwizera, C., et al. (2015). Sigma-1 agonist binding in the aging rat brain: A MicroPET study with [11C]SA4503. Mol. Imaging Biol. 18, 588–597. doi: 10.1007/s11307-015-0917-6

Ravasi, L., Tokugawa, J., Nakayama, T., Seidel, J., Sokoloff, L., Eckelman, W., et al. (2012). Imaging of the mu-synaptic acetylcholine neuroreceptor in rats with the M2 selective agonist [18F] FP-TZTP. Nucl. Med. Biol. 39, 45–55. doi: 10.1016/j.nucmedbio.2011.06.003

Ravert, H., Mathews, W., Musachio, J., Scheffel, U., Finley, P., and Dannals, R. (1999). [11C]methyl 4-[(3,4-dichlorophenyl)acetyl]N-[1-pyrrolidinylmethyl]1-piperazinecarboxylate. Nucl. Med. Biol. 26, 737–741. doi: 10.1016/s0969-0851(03)00285-2

Ray, R., Ruparel, K., Newberg, A., Wileyto, E., Loughead, J., Divgi, C., et al. (2011). Imaging dopamine D3 receptors in the human brain with positron emission tomography, [11C]PHNO, and a selective D3 receptor antagonist. Biol. Psychiatry 68, 392–399. doi: 10.1016/j.biopsych.2010.04.038

Seeman, P. (2009). Dopamine D2 High receptors measured ex vivo are elevated in amphetamine-sensitized animals. Synapse 63, 186–192. doi: 10.1002/syn.20595

Seeman, P. (2012). Dopamine agonist radioligand binds to both D2High and D2Low receptors, explaining why alterations in D2High are not detected in human brain scans. Synapse 66, 88–93. doi: 10.1002/syn.20987

Seeman, P., Hall, F. S., and Uh1. G. (2007). Increased dopamine D2 High receptors in knockouts of the dopamine transporter and the vesicular monoamine transporter may contribute to spontaneous hyperactivity and dopamine supersensitivity. Synapse 61, 573–576. doi: 10.1002/syn.20402

Shen, S., Finnema, S., Farde, L., Gulyás, B., Wikström, H., Halldin, C., et al. (2006). Effect of amphetamine on dopamine D2 receptor binding in nonhuman primate brain: a comparison of the agonist radioligand [11C]MNPA and antagonist [11C]clopenthixol. Synapse 59, 260–269. doi: 10.1002/syn.20238

Shen, N., Skineberg, M., Zogghi, S. S., Liow, J., Gladding, R. L., Hong, J., et al. (2008a). Kinetic brain analysis and whole-body imaging in monkey of [11C]MNPA: a dopamine agonist radioligand. Synapse 62, 700–709. doi: 10.1002/syn.20544

Shen, N., Zogghi, S., Skineberg, M., Liow, J., Hong, J., Sibley, D., et al. (2008b). Occupancy of dopamine D2/3 receptors in rat brain by endogenous dopamine measured with the agonist positron. Emiss. Tomogr. Radioligand 62, 756–763. doi: 10.1002/syn.20549

Shalgunov, D. (2017). Development of 18F-Labeled Agonist Radioligands for PET Imaging of the High-Affinity State of Cerebral Cerebral Dopamine D2/3 Receptors. Master's thesis, Groningen: University of Groningen.

Shalgunov, V., van Waarde, A., Booij, J., Michel, M. C., Dierckx, R. A. J. O., and Elsinga, P. H. (2019). Hunting for the high-affinity state of G-protein-coupled receptors with agonist tracers: theoretical and practical considerations for positron emission tomography imaging. Med. Res. Rev. 39, 1014–1052. doi: 10.1002/med.21552

Shen, C., Li, H., and Keller, E. (2002). Repeated treatment with antidepressants differentially alters 5-HT1A agonist-stimulated [35S]GTPγS binding in rat brain regions. Neuropharmacology 42, 1031–1038. doi: 10.1016/S0028-3908(02)00064-3

Shimoji, K., Esaki, T., Itoh, Y., Ravasi, L., Cook, M., Jehle, J., et al. (2003). Inhibition of [18F]FP-TZTP binding by loading doses of muscarinic agonists P-TZTP or FP-TZTP in vivo is not due to agonist-induced reduction in cerebral blood flow. Synapse 50, 151–163. doi: 10.1002/syn.10257

Shiue, C.-Y., Bai, L.-Q., Teng, R.-R., Arnett, C. D., Dewey, S. L., Wolf, A. P., et al. (1999). A comparison of the brain uptake of N-(cyclopropyl)[14C]methyl norethroporphine ([11C]buprenorphine) and N-(cyclopropyl)[14C]methyl nordiprenorphine ([11C]diprenorphine) in baboon using PET. Int. J. Radiat. Appl. Instrum. Part B Nucl. Med. Biol. 18, 281–288. doi: 10.1016/0887-2897(91)90123-3

Schreiber, G., and Avisar, S. (2000). G proteins as a biochemical tool for diagnosis and monitoring treatments of mental disorders. Isr. Med. Assoc. J. 2(Suppl.), 86–91.

Schreiber, G., Golan, M., and Avisar, S. (2009). Beta-arrestin signaling complex as a target for antidepressants and as a depression marker. Drug News Perspect. 22, 467–480.

Scott, D. J., Domino, E. F., Heitzeg, M. M., Koepp, R. A., Ni, L., Guthrie, S., et al. (2007a). Smoking modulation of µ-Opioid and dopamine D2 receptor-mediated neurotransmission in humans. Neuropsychopharmacology 32, 450–457. doi: 10.1038/sj.npp.1310238

Scott, D. J., Stohler, C. S., Koepp, R. A., and Zubieta, J.-K. (2007b). Time-course of change in [11C]carfentanil and [11C]raclopride binding potential after a nonpharmacological challenge. Synapse 61, 707–714. doi: 10.1002/syn.20404

Scott, D. J., Stohler, C. S., Egnatuk, C. M., Wang, H., Koepp, R. A., and Zubieta, J.-K. (2008). Placebo and nocebo effects are defined by opposite opioid and dopaminergic responses. Arch. Gen. Psychiatry 65, 220–231. doi: 10.1001/archpsyc.2007.34

Searle, G., Beaver, I. D., Comley, R. A., Bani, M., Tziortzi, A., Slifstein, M., et al. (2010). Imaging dopamine D3 receptors in the human brain with positron emission tomography, [11C]PHNO, and a selective D3 receptor antagonist. Biol. Psychiatry 68, 392–399. doi: 10.1016/j.biopsych.2010.04.038

Scott, D. J., Stohler, C. S., Egnatuk, C. M., Wang, H., Koepp, R. A., and Zubieta, J.-K. (2008). Placebo and nocebo effects are defined by opposite opioid and dopaminergic responses. Arch. Gen. Psychiatry 65, 220–231. doi: 10.1001/archpsyc.2007.34
Talbot, P. S., Narendran, R., Butelman, E. R., Huang, Y., Ngo, K., Slifstein, M., Colom et al. Agonists in PET Neuroimaging

Thorell, J. O. (1995). (R)-[N-11C-methyl]11-hydroxy-10-methylaporphine as a tracer for striatal D2 receptor occupancy. Life Sci. 46, 511ñ517. doi: 10.1016/j.lfs.2008.12.015

Vandecallepele, M., Dumont, F., De Vos, F., Strijckmans, K., Leysen, D., Audenaert, K., et al. (2004). Synthesis and preliminary in vivo evaluation of 4-[18F]fluoro-N-2-[4-(6-trifluoromethylpyridin-2-yl)piperazin-1-yl]ethylbenzamide, a potential PET radioligand for the 5-HT1A receptor. J. Label. Compd. Radiopharm. 47, 531ñ542. doi: 10.1002/jlcr.837

Vidal, B., Siebti, J., Verdurand, M., Fieux, S., Billard, T., Streichenberger, N., et al. (2016). Agonist and antagonist bind differently to 5-HT1A receptors during Alzheimer's disease: a post-mortem study with PET radiopharmacuticals. Neuropharmacology 109, 88ñ95. doi: 10.1016/j.neuropharm.2016.05.009

Wager, T. D., Scott, D. J., and Zubieta, J.-K. (2007). Placebo effects on human mu-opioid activity during pain. Proc. Natl. Acad. Sci. U.S.A. 104, 11056ñ11061. doi: 10.1073/pnas.0812305105

Watson, J., Collin, L., Ho, M., Riley, G., Scott, C., Selkirk, J. V., et al. (2000). 5-[11C]-(+)-PHNO and [11C]-(+)-MNPA binding to striatal D2/D3 dopamine receptors: A PET study in conscious monkeys. Synapse 45, 207ñ212. doi: 10.1002/syn.10102

Wada, J., Weerts, E. M., Kuwabora, H., Wong, D. F., Xu, X., and McAuley, M. E. (2012). The relationship between nalozone-induced cortical and mu opioid receptor availability is disrupted in alcohol dependent subjects. Alcohol. Clin. Exp. Res. 36, 511ñ517. doi: 10.1111/j.1530-0270.2011.01306.x

Wagner, J. M. (2014). A Philosophy for CNS radiotracer design. Acc. Chem. Res. 47, 3127ñ3134. doi: 10.1021/ar500233s

van der Werf, J. F., Sebens, J. B., Vaalburg, W., and Korf, J. (1983). In vivo binding of N-propylnorapomorphine in the rat brain: regional localization, quantification in striatum and lack of correlation with dopamine metabolism. Eur. J. Pharmacol. 87, 259ñ270. doi: 10.1016/0014-2999(83)90336-9

van Laere, K., Casteels, C., Lunsken, S., Goffin, K., Grachev, I. D., Bormans, G., et al. (2012). Regional changes in type 1 cannabinoid receptor availability in Parkinson’s disease in vivo. Neurobiol. Aging 33, 620ñ628. doi: 10.1016/j.neurobiolaging.2011.02.009

van Laere, K., Koole, M., Sanabria Bohorquez, S., Goffin, K., Guether, I., Belanger, M., et al. (2008b). Whole-body biodistribution and radiation dosimetry of the human cannabinoid type-1 receptor ligand 18F-MK-9470 in healthy subjects. J. Nucl. Med. 49, 439ñ445. doi: 10.2967/jnumed.107.047290

van Laere, K., Sanabria-Bohorquez, S., Mozley, D., Burns, D., Hamill, T., Van Hecken, A., et al. (2013). 11C-MK-8278 PET as a tool for pharmacodynamic brain occupancy of histamine 3 receptor inverse agonists. J. Nucl. Med. 55, 65ñ72. doi: 10.2967/jnumed.113.122515

van Laere, K. J., Sanabria-Bohorquez, S. M., Mozley, D. P., Burns, D. H., Hamill, T. G., Van Hecken, A., et al. (2014). 11C-MK-8278 PET as a tool for pharmacodynamic brain occupancy of histamine 3 receptor inverse agonists. J. Nucl. Med. 55, 65ñ72. doi: 10.2967/jnumed.113.122515

van Oosten, E. W., Wilson, A. A., Stephenson, K. A., Mamo, D. C., Pollock, B. G., Mulsant, B. H., et al. (2009). An improved radiosynthesis of the muscarinic M2 radiopharmaceutical, [18F]FP-TZTP. Appl. Radiat. Isot. 67, 611ñ616. doi: 10.1016/j.apradiso.2008.05.030

Vandecallepele, M., Dumont, F., De Vos, F., Strijckmans, K., Leysen, D., Audenaert, K., et al. (2004). Synthesis and preliminary in vivo evaluation of 4-[18F]fluoro-N-2-[4-(6-trifluoromethylpyridin-2-yl)piperazin-1-yl]ethylbenzamide, a potential PET radioligand for the 5-HT1A receptor. J. Label. Compd. Radiopharm. 47, 531ñ542. doi: 10.1002/jlcr.837

Vidal, B., Fieux, S., Redouté, J., Villien, M., Bonnefoi, F., Le Bars, D., et al. (2018). In vivo biased agonism at 5-HT1A receptors: characterisation by simultaneous PET/MR imaging. Neuropsychopharmacology 43, 2310ñ2319. doi: 10.1038/s41386-018-0145-2

Vidal, B., Sebti, J., Verdurand, M., Fieux, S., Billard, T., Streichenberger, N., et al. (2016). Agonist and antagonist bind differently to 5-HT 1A receptors during Alzheimer’s disease: a post-mortem study with PET radiopharmacuticals. Neuropharmacology 109, 88ñ95. doi: 10.1016/j.neuropharm.2016.05.009

Wager, T. D., Scott, D. J., and Zubieta, J.-K. (2007). Placebo effects on human mu-opioid activity during pain. Proc. Natl. Acad. Sci. U.S.A. 104, 11056ñ11061. doi: 10.1073/pnas.0702413104

Wand, G. S., Weerts, E. M., Kuwabora, H., Wong, D. F., Xu, X., and McAuley, M. E. (2012). The relationship between nalozone-induced cortical and mu opioid receptor availability is disrupted in alcohol dependent subjects. Alcohol. Clin. Exp. Res. 36, 511ñ517. doi: 10.1111/j.1530-0270.2011.01306.x

Watson, J., Collin, L., Ho, M., Riley, G., Scott, C., Selkirk, J. V., et al. (2000). 5-HT1A receptor agonist-antagonist binding affinity difference as a measure of

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intrinsic activity in recombinant and native tissue systems. *Br. J. Pharmacol.* 130, 1108–1114. doi: 10.1038/sj.bjp.0703394

Weerts, E. M., Kim, Y. K., Wand, G. S., Dannals, R. F., Lee, J. S., Frost, J. J., et al. (2008). Differences in delta- and mu-opioid receptor blockade measured by positron emission tomography in naltrexone-treated recently abstinent alcohol-dependent subjects. *Neuropsychopharmacol. Off. Publ. Am. Coll. Neuropsychopharmacol.* 33, 653–665. doi: 10.1038/sj.npp.1301440

Weerts, E. M., Wand, G. S., Kuwabara, H., Munro, C. A., Dannals, R. F., Hilton, J., et al. (2011). Positron emission tomography imaging of mu- and delta-opioid receptor binding in alcohol-dependent and healthy control subjects. *Alcohol Clin. Exp. Res.* 35, 733–742. doi: 10.1111/j.1530-0270.2012.12022

Willeit, M., Ginovart, N., Graff, A., Rusjan, P., Vitcu, I., Houle, S., et al. (2008). First human evidence of d-amphetamine induced displacement of a D2/D3 agonist radioligand: a [11C]-(+)-PHNO positron emission tomography study. *Neuropsychopharmacology* 33, 279–289. doi: 10.1038/sj.npp.1301400

Willeit, M., Ginovart, N., Kapur, S., Houle, S., Hussey, D., Seeman, P., et al. (2006). High-Affinity states of human brain dopamine D2/D3 receptors imaged by the agonist [11C]-(+)-PHNO. *Biol. Psychiatry* 59, 389–394. doi: 10.1016/j.biopsych.2005.09.017

Wilson, A. A., McCormick, P., Kapur, S., Willeit, M., Garcia, A., Hussey, D., et al. (2005). Radiosynthesis and evaluation of [11 C]-(+)-4-Propyl-3,4,4a,5,6,10b-hexahydro-2 H-naphthol[1,2- b][1,4]oxazin-9-ol as a potential radiotracer for in vivo imaging of the dopamine D2 high-affinity state with positron emission tomography. *J. Med. Chem.* 48, 4153–4160. doi: 10.1021/jm050155n

Wong, D., Kuwabara, H., Horti, A., Raymont, V., Brasic, J., Guevara, M., et al. (2010). Quantification of cerebral cannabinoid receptors subtype 1 (CB1) in healthy subjects and schizophrenia by the novel PET radioligand [11C]OMAR. *Neuroimage* 52, 1505–1513. doi: 10.1016/j.neuroimage.2010.04.034

Wong, D. F., Kuwabara, H., Horti, A. G., Kumar, A., Brasic, J., Ye, W., et al. (2008). “Imaging of Human Cannabinoid CB1 Type Human Receptors with [11C]OMAR,” in Proceedings of the 55th Annual Meeting of the Society of Nuclear Medicine, New Orleans, LA.

Yang, K.-C., Stepnov, V., Martinsson, S., Ettrup, A., Takano, A., Knudsen, G. M., et al. (2017). Fenfluramine reduces [11C]Cimbi-36 binding to the 5-HT2A receptor in the nonhuman primate brain. *Int. J. Neuropsychopharmacol.* 20, 683–691. doi: 10.1093/ijn/pxy051

Yokoyama, C., Mawatari, A., Kawasaki, A., Takeda, C., Onoe, K., Doi, H., et al. (2016). Marmoset serotonin 5-HT 1A receptor mapping with a biased agonist PET probe 18 F-F13714: comparison with an antagonist tracer 18 F-MPPF in awake and anesthetized states. *Int. J. Neuropsychopharmacol.* 19:yw079. doi: 10.1093/ijn/pwy079

Zijlstra, S., van der Worp, H., Wiegman, T., Visser, G. M., Korf, J., and Vlaeburg, W. (1993). Synthesis and in vivo distribution in the rat of a dopamine agonist: N-[(11C)methyl]norapomorphine. *Nucl. Med. Biol.* 20, 7–12. doi: 10.1016/0969-8051(93)90131-d

Zimmer, L. (2016). Pharmacological agonists for more-targeted CNS radio-pharmaceuticals. *Oncotarget* 7, 8011-8012. doi: 10.18632/oncotarget.13418

Zimmer, L., Fournet, G., Benoit, J., Guillaumet, G., and Le Bars, D. (2003). Carbon-11 labelling of 8[[3-[4-(2-[[11C]methoxyphenyl]piperazin-1-y]l]-2-hydroxypropyl]oxy]thiochroman, a presynaptic 5-HT(1A) receptor agonist, and its in vivo evaluation in anaesthetised rat and in awake cat. *Nucl. Med. Biol.* 30, 541–546. doi: 10.1016/s0969-8051(03)00271-7

Zimmer, L., Mauger, G., Le Bars, D., Bonmarchand, G., Luxen, A., and Pujol, J.-F. (2002). Effect of endogenous serotonin on the binding of the 5-HT1A PET ligand [11 F]MPPF in the rat hippocampus: kinetic beta measurements combined with microdialysis. *J. Neurochem.* 80, 278–286. doi: 10.1046/j.0022-3042.2001.00696.x

Zimmer, L., Riad, M., Rabah, L., Belkacem-Kahlouli, A., Le Bars, D., Renaud, B., et al. (2004). Toward brain imaging of serotonin 5-HT1A autoreceptor internalization. *Neuroimage* 22, 1421–1426. doi: 10.1016/j.neuroimage.2004.03.020

Zubieta, J., Dannals, R., and Frost, J. (1999). Gender and age influences on human mu-opioid receptor binding measured by PET. *Am. J. Psychiatry* 156, 842–848. doi: 10.1176/ajp.156.6.842

Zubieta, J., Gorelick, D., Stauffer, R., Ravert, H., Dannals, R., and Frost, J. (1996). Increased mu opioid receptor binding detected by PET in cocaine–dependent men is associated with cocaine craving. *Nat. Med.* 2, 1225–1229. doi: 10.1038/nm1196-1225

Zubieta, J., Greenwald, M. K., Lombardi, U., Woods, J. H., Kilbourn, M. R., Jewett, D. M., et al. (2000). Buprenorphine-induced changes in mu-opioid receptor availability in male heroin-dependent volunteers: a preliminary study. *Neuropsychopharmacol. Off. Publ. Am. Coll. Neuropsychopharmacol.* 23, 326–334. doi: 10.1016/S0893-133X(00)01010-X

Zubieta, J.-K., Bueller, J. A., Jackson, L. R., Scott, D. J., Xu, Y., Koepp, R. A., et al. (2005). Placebo effects mediated by endogenous opioid activity on mu-opioid receptors. *J. Neurosci. Off. J. Soc. Neurosci.* 25, 7754–7762. doi: 10.1523/JNEUROSCI.0439-05.2005

**Conflict of Interest:** The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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