

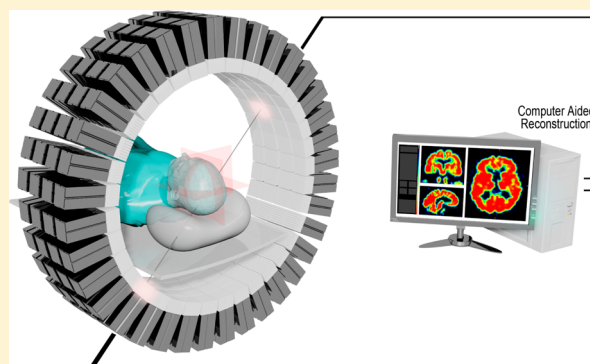
# Positron Emission Tomography in CNS Drug Discovery and Drug Monitoring

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**ABSTRACT:** Molecular imaging methods such as positron emission tomography (PET) are increasingly involved in the development of new drugs. Using radioactive tracers as imaging probes, PET allows the determination of the pharmacokinetic and pharmacodynamic properties of a drug candidate, via recording target engagement, the pattern of distribution, and metabolism. Because of the noninvasive nature and quantitative end point obtainable by molecular imaging, it seems inherently suited for the examination of a pharmaceutical's behavior in the brain. Molecular imaging, most especially PET, can therefore be a valuable tool in CNS drug research. In this Perspective, we present the basic principles of PET, the importance of appropriate tracer selection, the impact of improved radiopharmaceutical chemistry in radiotracer development, and the different roles that PET can fulfill in CNS drug research.



## ■ INTRODUCTION

Modern medical imaging techniques have become important tools for the early diagnosis and therapy management of various diseases of the central nervous system (CNS) and other organs. Morphological imaging techniques, e.g., computed tomography (CT) or magnetic resonance imaging (MRI), provide valuable information on biological structures or anatomy, whereas functional or spectroscopic MRI provides certain types of information about brain function. In contrast, molecular imaging techniques such as positron emission tomography (PET) and single-photon emission computed tomography (SPECT), through the detection of radiotracer molecules, allow the visualization of biological processes in the living organism.<sup>1</sup> The main processes of interest are cerebral blood flow, intra- and extracellular enzymatic reactions, which can reveal rates of metabolism or protein synthesis, and receptor–ligand interactions, which are relevant to studies of neurotransmission. Using these imaging modalities, it is possible to detect a pathophysiological state, arising from a deregulated biochemical process, before any discernible anatomical changes occur. Because of the high sensitivity of both modalities, PET and SPECT, their radiotracers can be applied in very small amounts. Hence, they are administered at doses usually devoid of any pharmacological effects, which prevents the perturbation of biological processes *in vivo*. PET is preferable to SPECT for many purposes, as PET recordings are quantifiable and are generally of higher sensitivity and better temporal and spatial resolution.<sup>2</sup>

For some neuropsychiatric disorders, PET and SPECT tracers serve as imaging probes to detect a disease (in particular,

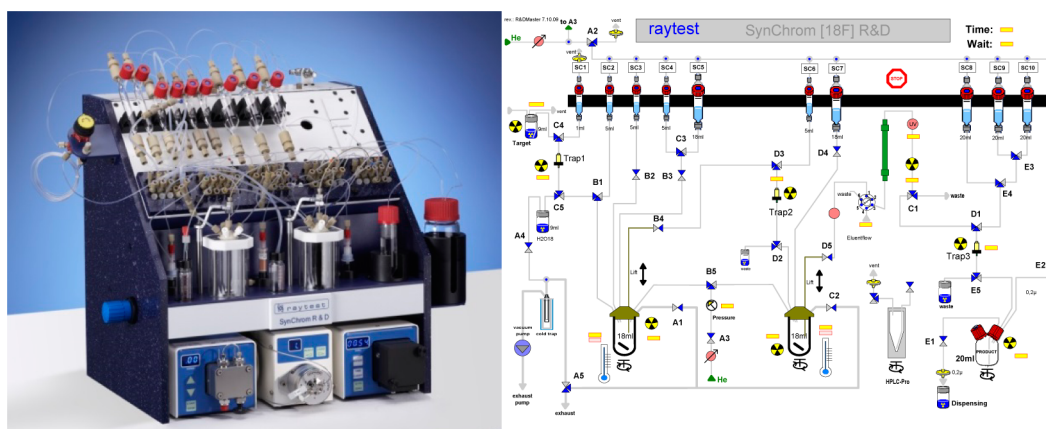
neurodegenerative disorders), but they can also be applied as a valuable tool in the development of new molecular entities or for the evaluation of the pharmacological behavior of drug candidates in a fast and efficient manner.

Neurodegenerative disorders as well as most mental disorders cause a strong and long-lasting burden of disease, reduce relevantly the quality of life, and finally are responsible for high economic losses. Hence, it is a crucial task to further improve our understanding of such illnesses, with an aim to develop better therapeutic strategies. Molecular imaging methods, especially PET, are playing an important role in addressing these concerns because they are the only available methods that enable molecule-specific results in the living brain.

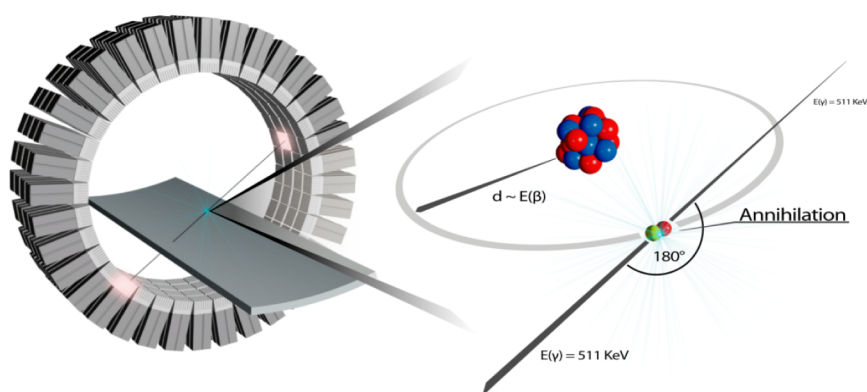
Our objective in this Perspective is to elucidate the principles of functional imaging with PET, the design of radiotracers for the CNS, their application in different strategies, and their roles in drug research. Furthermore, we link these contents with the real-life situation of PET in drug development, by reviewing this process based on the example of antipsychotic drug development: the use of PET in schizophrenia research and its association to antipsychotic drug development has a long history and covers all possible facets and pitfalls.

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**Figure 1.** Automated synthesis module (SynChrom R&D, RAYTEST) for the synthesis of  $^{18}\text{F}$ -labeled tracers (left). The programming and control of the different reaction steps is performed via a graphical interface (right).



**Figure 2.** Schematic diagram of a radionuclide emitting a positron, resulting annihilation, production of two 511 keV photons, and the coincident detection of the photons in a PET scanner.

## ■ PRINCIPLES OF POSITRON EMISSION TOMOGRAPHY

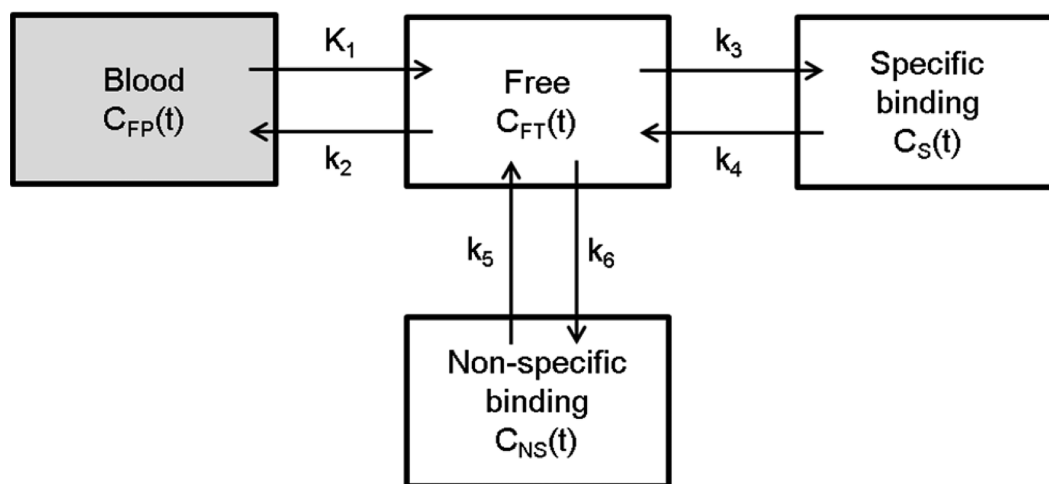
Most PET tracers consist of a pharmacophore and a positron-emitting radionuclide. While the pharmacophore is responsible for the pharmacological behavior of the tracer, the radionuclide provides the means for detection, which subsequently serves for source mapping, commonly known as imaging. The process of coupling the radionuclide to the pharmacophore is generally referred to as labeling or radiolabeling. Chemically, this is achieved via the formation of covalent bonds for nonmetallic radioisotopes (such as carbon, nitrogen, oxygen, and the halogens) and dative bonds whenever metallic radionuclides are concerned.

**Synthesis of Radiotracers.** Because of the generally short half-life of the neutron-deficient, positron-emitting radionuclides used for molecular imaging, these radionuclides usually have to be produced on a daily basis or even in the hour of intended use.<sup>3</sup> Particularly for the synthesis of radiotracers for biological targets in the living brain, a very high specific radioactivity (cf. choice of the radionuclide) of the radionuclide produced is often mandatory. The positron-emitting radionuclide is first generated with a cyclotron and then reacted with a suitable precursor, which is a molecule allowing the incorporation of the radionuclide in a fast and efficient manner. Radiolabeling is performed as late as possible in the synthetic procedure and should not entail many subsequent synthetic steps or complicated purification

protocols for isolating the product, since each passing minute reduces the final radioactivity of the labeled product. Because the precursor is used in large excess compared to the radionuclide, the unreacted precursor should be separated from the radiotracer to obtain high chemical purity and avoid pharmacological and/or toxic effects from carryover of the precursor. After purification of the tracer, it is usually formulated in isotonic sodium chloride solution or other suitable buffers, sometimes with the addition of up to 10% v/v ethanol as required for solubility. Finally, the formulated tracer undergoes quality control for important parameters such as purity (chemical, radiochemical and radionuclidic), pH, and sterility, as required by current good manufacturing practice (cGMP, see below) requirements for the preparation of products intended for intravenous administration.

For tracers routinely applied in human studies, the synthesis is often carried out using automated synthesis modules. These modules permit fully automated one- or, sometimes, two-step reactions, which not only increases the reliability of the synthesis but also reduces the radiation exposure of the staff. A typical automated synthesis module and schematic plan for the production of  $^{18}\text{F}$ -fluorinated tracers is shown in Figure 1.

Because of the short half-lives of the radionuclides and the requirements for radiation protection and cGMP, the synthesis and quality control of radiotracers for PET studies is a demanding task for the radiopharmacy. Thus, special procedures had to be developed for efficient synthesis, quality



**Figure 3.** Diagrammatic representation of the two-tissue compartment model, consisting of blood ( $C_{FP}$ ), free tracer in tissue ( $C_{FT}$ ), specific binding ( $C_S$ ) and nonspecific binding ( $C_{NS}$ ) compartments, and the blood–brain unidirectional clearance ( $K_1$ ), rate constant for clearance back to circulation ( $k_2$ ), and the relevant association and dissociation rate constants ( $k_3$ – $k_6$ ).

control, and formulation, which also contribute to a GMP-compliant tracer production.

**Regulatory Requirements.** Because radiotracers are considered to be drugs with respect to regulation, their production must follow the rules of cGMP. This guarantees a high standard for the quality of the radiotracer and also guarantees its chemical identity and purity. Hence, the synthesis and quality control must be performed using established and documented procedures and practices.<sup>4</sup> Furthermore, since December 2011, PET tracers must be manufactured in compliance with 21 Code of Federal Regulations Part (CFR) 212 in the U.S.<sup>5</sup> Consequently, all producers for PET tracers must be operating under an approved new drug application (NDA) or abbreviated new drug application (ANDA) for clinical tracers or under an investigational new drug application (IND) for research tracers.

**Positron Emission and PET Measurement.** The distinct advantage of PET over other molecular imaging methods is the higher degree of spatial and temporal resolution of recordings of the tracer's distribution and the possibility of quantifying its concentration as a function of time. These advantages arise from the special decay characteristics of positron-emitting radionuclides. The positron released from the decaying neutron-deficient radionuclide travels a short distance (typically ranging from 1 mm to 2 cm), depositing its kinetic energy in the surrounding matter, cf. Figure 2, in a process known as thermalization. After losing most of its kinetic energy, the antimatter positron can interact with its ordinary matter counterpart, an electron; annihilation of the two elementary particles releases their mass energy as radiation. The annihilation generates two photons of exactly 511 keV, emitted at almost 180° to each other.<sup>6</sup>

Coincident detection of these photons by a pair of radiation detectors on either side of the annihilation event provides the so-called line of response, which provides information about the approximate spatial location of the radionuclide. Modern PET scanners consist of several rings, each containing numerous detectors, which all are interconnected (cf. left side of Figure 2) by coincidence circuits. Upon counting of millions of annihilation events, a final source map can be calculated, typically with spatial resolution of 3–5 mm for contemporary human PET scanners and even <1 mm can be obtained for

small animal PET scanners ( $\mu$ PET scanners). In addition to the superior spatial resolution of those detector arrays, the detectors also possess dead times on the order of nano- to picoseconds. Because of statistical requirements and other factors, this property allows dynamic PET measurements of tracers in a temporal resolution of seconds.

Because even photons are scattered in condensed matter, a transmission scan is performed to determine correction factors for this scattering, which are then used to correct the emission scan for signal loss. The combination of transmission and emission scan allows an absolute quantification of the measured activity (becquerels per milliliter), which corresponds to the concentration of the tracer in the tissue.

**Processing of PET Data and Compartmental Modeling.** In research studies, most of the emission scans are still acquired as a dynamic series, in the manner of a motion picture. Here, as series of static recordings is separated in so-called time frames, each of which depicts a map of the activity concentration in the tissue in absolute units of kilobecquerels per milliliter for a certain time interval after tracer injection (usually in incrementally increasing time frame lengths). The definition of time frames depends on the tissue kinetics of the ligand used; if kinetics are unknown, then the storage and acquisition of event-by-event data (list mode) provides much better flexibility in the posthoc definition of dynamic recording frames and also improves the possibility for movement correction.<sup>7</sup> Indeed, in cases of long acquisition times lasting up to several hours, head movements are very frequent and pronounced, even when using head fixation systems (e.g., vacuum masks). When using predefined time frames, slow shifts in head position can be corrected by mathematical algorithms, whereas rapid intraframe movements are not amenable to this kind of correction, unless list-mode data is available. Another problem can arise from the paucity of anatomical information within the PET-recordings. Whereas ligands with high cortical uptake (e.g., 2-deoxy-2-[<sup>18</sup>F]fluoro-D-glucose, 2-[<sup>18</sup>F]FDG<sup>8</sup>) provide sufficient anatomical information for motion correction between frames, this can be difficult for late frame recordings of other tracers characterized by substantial washout from cortex, i.e., dopamine D<sub>2/3</sub>-receptor ligands. PET/CT and PET/MRI hybrid systems can be helpful in this event. In any event, PET-to-MRI coregistrations (e.g., to ICBM 452 templates) serve to



**Figure 4.** Images of a human PET/CT-Scanner (Discovery PET/CT 610; GE Healthcare) and a preclinical  $\mu$ PET/MRI-Scanner (nanoScanPM; Mediso Medical Imaging Systems).

**Table 1. Physical Properties and Production Routes of Important Radionuclides Usable for PET Imaging of the Central Nervous System<sup>15</sup>**

nuclide	half-life $t_{1/2}$	decay mode <sup>a</sup>	production	product	maximum specific activity (GBq/ $\mu$ mol)
<sup>15</sup> O	2.04 min	$\beta^+$ (99.9%) EC (0.01%)	<sup>15</sup> N(d,n) <sup>15</sup> O	[ <sup>15</sup> O] <sub>2</sub>	$3.4 \times 10^6$
<sup>11</sup> C	20.39 min	$\beta^+$ (99.8%) EC (0.2%)	<sup>14</sup> N(p, $\alpha$ ) <sup>11</sup> C	[ <sup>11</sup> C]CO <sub>2</sub> [ <sup>11</sup> C]CH <sub>4</sub>	$3.4 \times 10^5$
<sup>13</sup> N	9.97 min	$\beta^+$ (99.8%) EC (0.2%)	<sup>16</sup> O(p, $\alpha$ ) <sup>13</sup> N	[ <sup>13</sup> N]NH <sub>3</sub>	$7.0 \times 10^5$
<sup>18</sup> F	109.8 min	$\beta^+$ (96.7%) EC (3.3%)	<sup>18</sup> O(p,n) <sup>18</sup> F <sup>20</sup> Ne(d, $\alpha$ ) <sup>18</sup> F	[ <sup>18</sup> F]F <sup>-</sup> <sub>aq</sub> [ <sup>18</sup> F]F <sub>2</sub>	$6.3 \times 10^4$
<sup>76</sup> Br	16.0 h	$\beta^+$ (54.0%) EC (46.0%)	<sup>75</sup> As( <sup>3</sup> He,3n) <sup>76</sup> Br <sup>76</sup> Se(p,n) <sup>76</sup> Br	[ <sup>76</sup> Br]Br <sup>-</sup>	$7.2 \times 10^3$
<sup>124</sup> I	4.17 days	$\beta^+$ (22.8%) EC (11.0%)	<sup>124</sup> Te(p,n) <sup>124</sup> I	[ <sup>124</sup> I]I <sup>-</sup>	$1.2 \times 10^3$

<sup>a</sup> $\beta^+$ , positron emission; EC, electron capture

spatially normalize the dynamic PET recordings to standard spatial coordinates. Although nonlinear normalization algorithms have been improved in recent years, this step of PET data processing is one of the highest sources of bias, especially in cases with a differing extent of ventricular enlargement, as occurs in neurodegenerative disease.<sup>9</sup>

A key aspect of data PET analysis is to separate different pools of activity signals within the total signal. In particular, the activity detected by the tomograph might result from free/unbound tracer in brain together with a specifically bound component, as in the case of ligands for receptors or transporters or metabolically trapped ligands such as the glucose analogue 2-[<sup>18</sup>F]FDG or the dopamine synthesis tracer 6-[<sup>18</sup>F]fluoro-L-dopa ([<sup>18</sup>F]FDOPA).<sup>10</sup> In addition, kinetic analysis must consider activity derived from tracers in small blood vessels and capillaries in the tissue as well as radioactive metabolites in the blood compartment. Each of these sources of activity is defined by a specific component of a compartmental kinetic model. Ligand binding reactions can be considered reversible, such that bound tracer in brain is free to return to an unbound compartment. Kinetic models describing all of these processes can be highly complex and tend to be overspecified or unstable due to noisy data. For scientific purposes, it may be necessary to record not only the PET signal but also the tracer and its metabolite concentrations in samples of arterial plasma. Nevertheless, an overcomplicated model does not necessarily improve the interpretability of the results, as in the particularly complex case of [<sup>18</sup>F]FDOPA.<sup>11</sup> Usually, researchers employ a

two-tissue compartment model (cf. Figure 3), based on the arterial blood plasma ( $C_{FP}(t)$ ), and the total concentration in brain of free/unbound tracer in the tissue ( $C_{FT}(t)$ ) and tracer specifically bound to its biological target ( $C_S(t)$ ). These models necessarily simplify the real biological situation to a set of linear equations, by applying certain assumptions; solution of the equations either graphically or mathematically give estimates of the relevant rate constants and biological parameters, which are assumed to be constant for the duration of the PET recording. The typical outcome parameter is the binding potential ( $BP$ ), which is proportional to the ratio between the concentration of the binding site ( $B_{max}$ ) and the affinity of the ligand to this site (expressed as the dissociation constant  $K_D$ ) relative to the free fractions of the tracer in tissue or plasma. The term  $BP_p$  refers to  $BP$  relative to the free plasma concentration, which is calculated on the basis of analysis of arterial blood sampled during the PET recording. Blood sampling is frequently impractical, so specific binding can be calculated relative to uptake in a brain region with only nondisplaceable (ND) binding, which yields  $BP_{ND}$  as presented by Innis et al.<sup>12</sup> This outcome parameter is convenient to obtain, but it suffers from a caveat arising from the assumptions about the nonbinding reference region.

**Hybrid Systems.** Although PET gives important scientific and/or diagnostic information about biological processes, the addition of morphological imaging is essential for proper anatomic assignment and can simplify the modeling process. Hybrid systems for both clinical and preclinical imaging have

been developed to perform functional and morphological imaging in a single examination (cf. Figure 4). The detailed anatomical information provided by fusion imaging with PET/CT scanners dramatically improves the diagnostic accuracy of the PET scan.<sup>13</sup> Furthermore, the CT scan can be used for attenuation correction, thus sparing the subject an additional radiation exposure. Because proton MRI offers superior anatomical information to CT in soft tissues, hybrid PET/MRI scanners were recently developed and show great potential for increasing further the accuracy of PET for studying neurological conditions and brain abnormalities such as dementia, cerebrovascular disorders, or epilepsy.<sup>14</sup> The hybrid technology affords improved motion correction and the potential for simultaneous functional MRI acquisitions as well as MR-based attenuation correction, although this remains a technical challenge.

For a more comprehensive review of radioisotope production, radiopharmaceutical chemistry, PET imaging, and kinetic modeling, the reader is referred to recent reviews and books.<sup>15–19</sup>

## ■ DESIGN OF PET TRACERS FOR THE CENTRAL NERVOUS SYSTEM

**Choice of the Radionuclide.** The radionuclides can be generally divided into organic isotopes (representing the constituent elements of organic molecules), which are coupled to the pharmacophore via a covalent bond (e.g., <sup>18</sup>F and <sup>11</sup>C), and the metallic isotopes (e.g., <sup>68</sup>Ga), which are usually attached to a pharmacophore via a chelator. The nonmetallic positron-emitting radionuclides that have been used for visualization of processes in the CNS are listed in Table 1.

Besides this general differentiation between organic and metallic radionuclides, other properties relevant to the selection of the radionuclide include physical half-life, decay modes, production routes, specific activity, and chemical species obtained from the irradiation. These topics are discussed below:

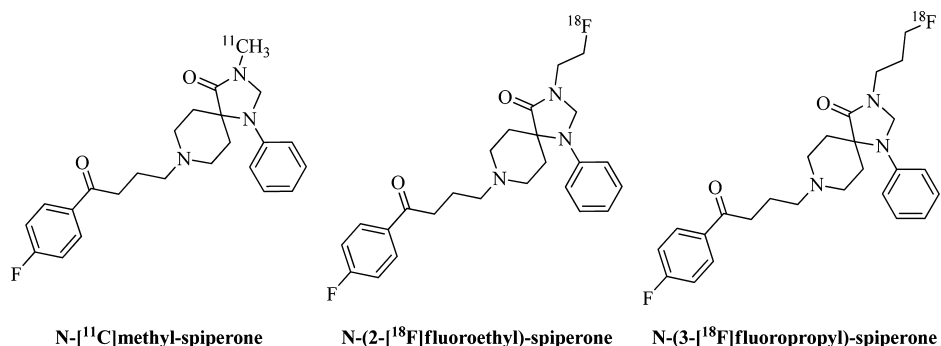
**Half-Life.** One of the most important physical properties of the radionuclide is its half-life, since it establishes the time-limit on the period available for scanning from a given radiosynthesis and thus also restricts possible radiosynthetic routes of the radiotracer. Very short-lived nuclides, e.g., <sup>15</sup>O ( $t_{1/2} = 2.04$  min), are inappropriate for performing complex preparations, and their applications are thus limited to very fast radiosyntheses and tracers with rapid kinetics. Because of their short half-lives, <sup>13</sup>N and <sup>15</sup>O can be used only for the synthesis of simple molecules, such as the perfusion tracers [<sup>15</sup>O]H<sub>2</sub>O or [<sup>13</sup>N]NH<sub>3</sub>; as a general rule, the production and quality control of a radiotracer should be finished within three half-lives to permit sufficient time for imaging. Furthermore, the half-life of the radionuclide should correlate with the biochemical process to be visualized, to allow a high enrichment of the tracer in the target region while retaining sufficient radioactivity for a quantitative determination of the tracer's distribution. Thus, <sup>11</sup>C ( $t_{1/2} = 20.39$  min) is generally well suited for studying the brain with small tracer molecules, but it is not suited for the investigation of antigen–antibody interactions, which have much slower kinetics; enrichment at the target area for antibodies typically requires several hours. On the other hand, using a long-lived radionuclide to investigate a rapid process will frequently result in an unnecessary radiation burden for the patient.

**Production Route.** Although not a physical property of the radionuclide itself, the production route represents an important factor for the logistics of radiochemistry and later application in PET imaging. A prominent example for the significance of the production route is <sup>18</sup>F-fluorine, which either can be produced via the <sup>18</sup>O(p,n)<sup>18</sup>F-reaction (producing nucleophilic [<sup>18</sup>F]F<sup>−</sup><sub>aq</sub>) or via the <sup>20</sup>Ne(d,α)<sup>18</sup>F-reaction (producing electrophilic [<sup>18</sup>F]F<sub>2</sub>). These routes result in different chemical species and consequently require different labeling strategies. Furthermore, the specific activities that can be obtained from these routes differ enormously, attaining more than 370 GBq/μmol for the <sup>18</sup>O(p,n)-reaction versus only 0.4 GBq/μmol for the <sup>20</sup>Ne(d,α)-reaction, with obvious implications for specific activity.

**Specific Activity.** An especially crucial parameter for CNS tracers is the specific activity, which is defined as the radioactivity per total mass of all isotopic compounds, typically expressed as a molar amount (gigabecquerels per micromole). A high specific activity not only avoids pharmacological or toxicological effects from the tracer but also is a crucial requirement for visualization of targets with a very low abundance or low density, such as dopamine D<sub>2/3</sub> receptors in nonstriatal regions. The importance of a high specific activity for CNS imaging, in particular for μPET studies, has been demonstrated recently for [<sup>11</sup>C]raclopride (3,5-dichloro-N-[(2S)-1-ethyl-2-pyrrolidinyl]methyl}-2-hydroxy-6-[<sup>11</sup>C]-methoxybenzamide).<sup>20</sup> If specific activity is too low, then significant occupancy of the binding site occurs, which reduces the magnitude of BP<sub>ND</sub>. In a rat PET study, tracer batches with a high specific activity (~150 GBq/μmol) gave a BP<sub>ND</sub> of about 2.5, whereas batches with a low specific activity (~150 MBq/μmol) gave a BP<sub>ND</sub> of only about 0.3, due to violation of the assumption for pharmacokinetic modeling that only a small fraction of the receptors is occupied by the tracer (typically <5%).<sup>12,21</sup> When using tracers of low specific activity, this assumption is no longer valid, and the coinjected unlabeled tracer blocks the receptor, thereby reducing the enrichment of the labeled compound. For preclinical studies, the specific activity is a very crucial point, since a much higher ratio of injected radioactivity/body weight is frequently used.

**Isotope and Analogue Tracers.** Because most physiologically interesting lead compounds consist of carbon, oxygen, and nitrogen, the positron-emitting radionuclides <sup>11</sup>C, <sup>13</sup>N, and <sup>15</sup>O are of particular interest. Tracers labeled by such an isotopic substitution, where the stable isotope of an organic element in the final radiotracer is replaced by a positron emitter, are known as isotope tracers. In this event, the chemical properties of the radiolabeled molecule do not differ from those of the nonlabeled, stable molecule. For <sup>18</sup>F or <sup>124</sup>I on the other hand, such an isotopic substitution is impossible if biomolecules and radiotracer candidates do not already contain fluorine or iodine or if the halogenated position is not accessible via typical labeling strategies. In this case, the initial molecules structure has to be chemically modified to allow a radiolabeling, which generates a derivative known as an analogue tracer.

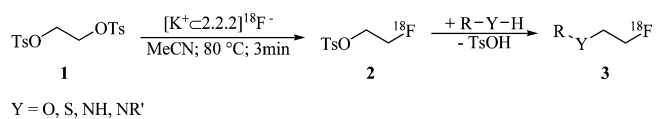
To minimize unwanted effects on the biological properties of the tracer, chemically similar exchanges are often utilized. For example, moieties with similar electronic (OH vs F) or steric (CH<sub>3</sub> vs I; H vs F) characteristics would be preferred for a substitution. In radiopharmacy, in contrast to the pharmaceutical research, these bioisosteric concepts are not used to improve the absorption, distribution, metabolism, or excretion (ADME) of drugs but to introduce the radioisotope at a



**Figure 5.** Structures of the dopamine and serotonin receptor antagonists  $N$ - $^{11}\text{C}$ methyl-spiperone (left),  $N$ - $(2$ - $^{18}\text{F}$ )fluoroethyl)-spiperone (middle), and  $N$ - $(3$ - $^{18}\text{F}$ )fluoropropyl)-spiperone (right).

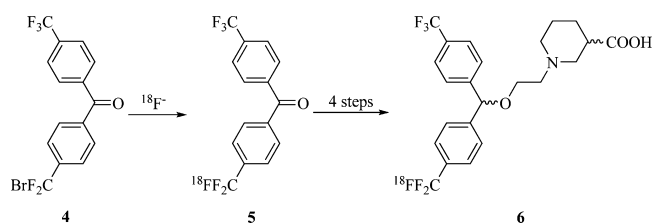
suitable position by taking into account the available labeling techniques. A high in vivo stability of the radioisotope, introduced via such a modification of the lead compound, is a major focus of this approach. However, such bioisosteric modifications seldom lead to an exact mimic of the original molecule. For example, an F-for-H exchange, due to the unique properties of fluorine, not only commonly results in an increased lipophilicity but also can influence the metabolism, conformation, membrane permeability, or pharmacological potency of the molecule in a manner unpredictable in direction and extent.<sup>22,23</sup> A prominent example for the examination of such modifications is  $N$ - $^{11}\text{C}$ methyl-spiperone (cf. Figure 5), an established radiotracer to study the dopaminergic and serotonergic neurotransmission in vivo.<sup>24</sup> Because of the short half-life of  $^{11}\text{C}$ , limiting the clinical application, different fluorinated analogues were examined as possible alternatives. Hence, different  $N$ -alkylated and  $N$ -fluoroalkylated derivatives were synthesized, and their properties were evaluated.<sup>25</sup> While a lower affinity for the dopamine receptor was observed for the  $N$ - $(2$ -fluoroethyl)-spiperone, in comparison with that for  $N$ -ethyl-spiperone, the  $N$ - $(3$ -fluoropropyl)-spiperone showed a higher affinity than the propyl-spiperone. Interestingly, this study also showed that the metabolites of  $N$ - $(2$ - $^{18}\text{F}$ )fluoroethyl)-spiperone were enriched in the brain, whereas for the metabolites of  $N$ - $(3$ - $^{18}\text{F}$ )fluoropropyl)-spiperone no significant brain uptake was observed. Thus,  $N$ - $(3$ - $^{18}\text{F}$ )fluoropropyl)-spiperone is the preferred  $^{18}\text{F}$ -labeled spiperone analogue for PET studies in vivo.

For the case of  $^{18}\text{F}$ , a special approach is frequently used for the introduction of the radioisotope. Lead structures containing methyl groups (such as  $\text{ROCH}_3$  or  $\text{R}_2\text{NCH}_3$ ) are generally not accessible via F vs H substitution, due to the metabolic instability of the resulting fluoromethyl compound, which is prone to defluorination. In these cases, the methyl group is often substituted by a fluoroethyl moiety, usually resulting in a more stable compound compared to that of the fluoromethyl derivative, with an affinity not significantly different than that of the lead compound. In contrast to direct  $^{18}\text{F}$ -fluorination,  $^{18}\text{F}$ -fluoroethylation is performed via a secondary labeling precursor. This term refers to very reactive small molecules accessible from the irradiation products in a fast and reliable manner. Thus, for example,  $^{18}\text{F}$ fluoride is reacted with a molecule consisting of an ethylene group and two leaving groups, usually ethylene ditsylate (1), giving the  $^{18}\text{F}$ -fluoroethylating agent. Subsequently, this secondary labeling precursor is reacted with the demethylated lead structure (the precursor), to obtain the  $^{18}\text{F}$ -fluoroethylated compound 3, cf. Figure 6.



**Figure 6.** Synthesis of 2- $^{18}\text{F}$ fluoroethyltosylate (2) and subsequent labeling step using this secondary labeling precursor.

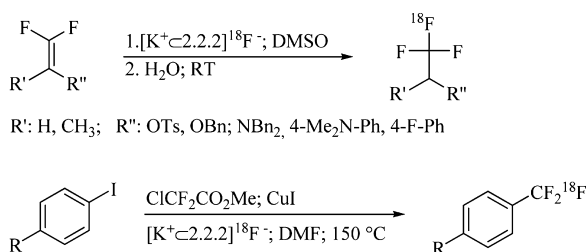
To avoid the need for pharmacological re-evaluation of modified compounds, isotopic labeling methods are generally preferred. To make this possible, a diverse number of procedures and reactions need to be developed to make all positions/atoms in a compound accessible for rapid and efficient isotopic labeling. This is even more crucial for elements seldom occurring in lead structures, such as fluorine or iodine. Fluorine is often present in drug candidate molecules as the trifluoromethyl group, because trifluoromethylation is a common approach for lead optimization in drug research. However,  $^{18}\text{F}$ -labeling of this moiety is usually accompanied by harsh reaction conditions, elaborate multistep reactions, and low reactivity of the leaving groups, which result in low radiochemical yields and/or low specific activities.<sup>26–28</sup> For example, the synthesis of 1-(2-((4- $^{18}\text{F}$ trifluoromethyl)(4-(trifluoromethyl)phenyl)methoxy)ethyl)piperidine-3-carboxylic acid (6, cf. Figure 7), a GABA uptake inhibitor, was prepared



**Figure 7.** Synthesis of the GABA uptake inhibitor 6 via  $^{18}\text{F}$ -direct fluorination of 4 in a five-step reaction sequence.

via an  $^{18}\text{F}$ -direct fluorination of 4 in a five-step reaction.<sup>26</sup> After optimization of the reaction sequence, the radiotracer 6 finally was obtained in radiochemical yields of 17–28% after a synthesis time of 150 min and with specific activities of about 74 GBq/ $\mu\text{mol}$ . Although this example shows that  $^{18}\text{F}$ -trifluoromethylated radiotracers can be synthesized, in principle, the disadvantages described above severely limit their application in clinical PET studies.

Recently, this problem was addressed by developing new approaches for the synthesis of aliphatic and aromatic  $^{18}\text{F}$ -labeled trifluoromethyl moieties (cf. Figure 8).<sup>29,30</sup> In

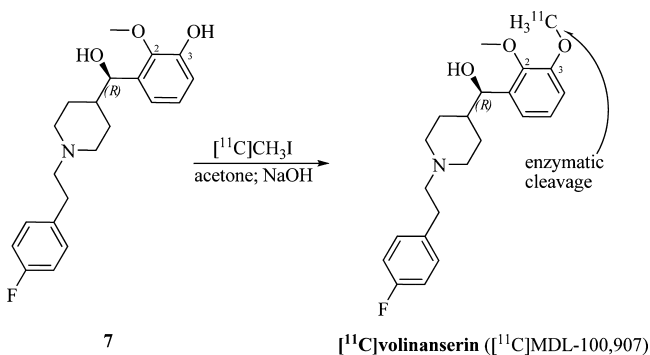


**Figure 8.** Production pathways for the synthesis of aliphatic (upper row) and aromatic (lower row)  $^{18}F$ -labeled trifluoromethyl moieties.

comparison to the previous methods, these new approaches avoid low radiochemical yields and insufficient specific activities, making the synthesis of [ $^{18}F$ ]trifluoromethyl moieties more attractive for routine applications.

**Labeling Position.** The position at which labeling occurs on the precursor is of crucial importance for CNS tracers. While this is necessarily true for analogue tracers, it also applies to isotope tracers, for which several positions in a lead structure can be considered for labeling. Because tracer metabolism often cannot be avoided, the position at which the radiolabel is introduced then determines which metabolites are radioactive and which are not. Only metabolites with the radionuclide attached will still be visible in a PET scan. If, for example, the radioactive metabolite possesses a moderate affinity for the target and enriches there, then its formation may hamper evaluation of the PET signal. Consequently, polar radioactive metabolites are preferred, because these either fail to cross the blood–brain barrier (BBB) or are rapidly washed out from brain and therefore do not contribute importantly to the recording. For example, defluorination of  $^{18}F$ -fluorinated tracers releases anionic [ $^{18}F$ ]fluoride, which is rapidly cleared from the brain. This can result in labeling of the skull during longer scans and can be a significant factor interfering in the quantitation of small animal PET scans.<sup>31</sup>

An illustrative example for the importance of the labeling position is provided by the synthesis of the 5-HT<sub>2A</sub>-ligand [ $^{11}C$ ]volinanserin ([ $^{11}C$ ]MDL-100,907).<sup>32</sup> In this case,  $^{11}C$ -methylation is a preferred reaction for introducing the  $^{11}C$ -label, for which volinanserin offers two different positions: the 2- and the 3-position of the aromatic ring (cf. Figure 9). In previous studies, it was shown that volinanserin is partly metabolized to its 3-OH analogue. Hence,  $^{11}C$ -methylation in the 2-position would result in the formation of a lipophilic  $^{11}C$ -labeled metabolite, which can be expected to enter the brain and potentially increase nonspecific binding or indeed bind to receptors. In contrast,  $^{11}C$ -methylation at the 3-position of the



**Figure 9.** Labeling reaction and metabolization of [ $^{11}C$ ]volinanserin.

precursor 7 results in the formation of polar metabolites, such that selection of this labeling position is much preferable.

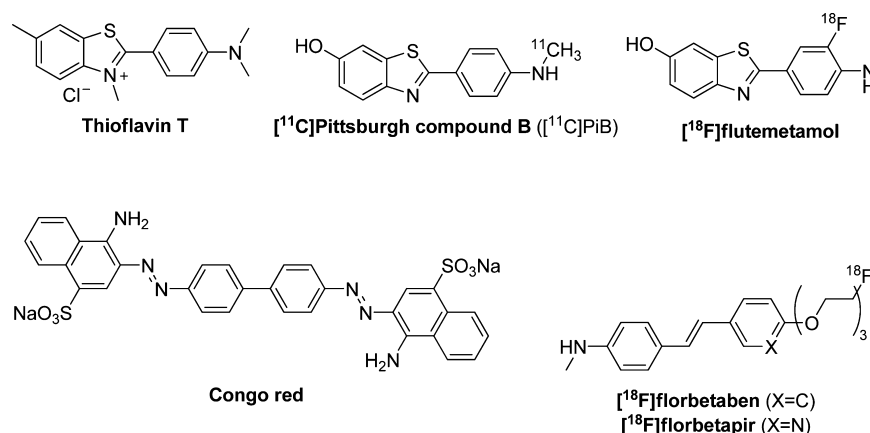
**Design of the Pharmacophore.** In addition to the choice of radionuclide, the properties of the pharmacophore also significantly influence functional imaging. Hence, some essential aspects of the pharmacophore, including its permeability across the BBB, its binding affinity and selectivity for the target, and its vulnerability to metabolism (noted above), have to be considered.

**Permeability.** In order to reach the CNS, tracers need to cross the BBB. Lipinski et al. developed a set of empirical rules predicting BBB permeability, which are based on rough estimates of lipid solubility and membrane penetration.<sup>33</sup> The so-called Lipinski rule of five, which is based on a database of the properties of ~2500 compounds, states that compounds with a log P (logarithm of the partition coefficient octanol/water) less than 5, a molecular weight less than 500, five or fewer hydrogen-bond donors (HBD), and 10 or fewer hydrogen-bond acceptors generally show good entry into brain.<sup>33</sup> Although originally developed for the oral bioavailability of drugs, these rules are applicable for the case of CNS PET tracers. Low BBB permeability remains one of the most frequent causes for the failure of promising CNS tracers and recently motivated the development of a prediction model specifically for CNS PET tracers. This method incorporates calculated distribution coefficients at pH 7.4 (cLog D<sub>7.4</sub>), molecular weight, the topological polar surface area (TPSA), the number of hydrogen-bond donors (HBD), and the protonation constant pK<sub>a</sub> of the most basic center.<sup>34</sup> Prediction models are important tools for the identification of promising structure–transport correlations and can spare a resource-intensive and expensive development of tracers ultimately proving to have unfavorable properties. While low lipophilicity of the tracer reduces BBB permeability, very high lipophilicity results in excessive nonspecific binding, imparting high background signal, which makes the quantitation of the specific binding signal difficult. Furthermore, nonspecific binding increases the radiation burden to the patient.

The uptake of tracers into the CNS can also be restricted by a number of efflux transporters located in the BBB.<sup>35</sup> P-glycoprotein (P-gp), the most prominent of these, occurs at the luminal side of brain capillary endothelium cells and acts as an active (ATP-driven) efflux pump for a wide range of compounds. Hence, cerebral permeability of CNS tracers that are substrates of P-gp is attenuated, which can be a source of variability of tracer uptake and binding.<sup>36</sup> A number of studies, both in vitro and in vivo, have been performed recently to examine possible interactions of established radiotracers and P-gp.<sup>37</sup>

**Affinity and Selectivity.** Most brain targets are expressed at very low levels, which demand a very high affinity of the radiotracer for its particular target. Because PET tracers, in contrast to normal pharmaceuticals, are typically administered in submicrogram amounts, a high affinity is especially crucial, as the radiotracer often has to compete with endogenous ligands for the binding sites of their common target. CNS PET tracers should preferably show an affinity for their target in the low nanomolar to subnanomolar range. As mentioned before, the binding potential primarily depends on  $B_{max}$  and  $K_D$ . Thus, lower  $K_D$  (higher affinity at the target) increase these values.

Equally important is a high selectivity for the target, which results in fewer specific interactions with other binding sites, such as other receptors, receptor subtypes, or transporters. The



**Figure 10.** Structures of the lead compounds for the diagnosis of Alzheimer's disease and the corresponding  $^{18}\text{F}$ -fluorinated tracers.

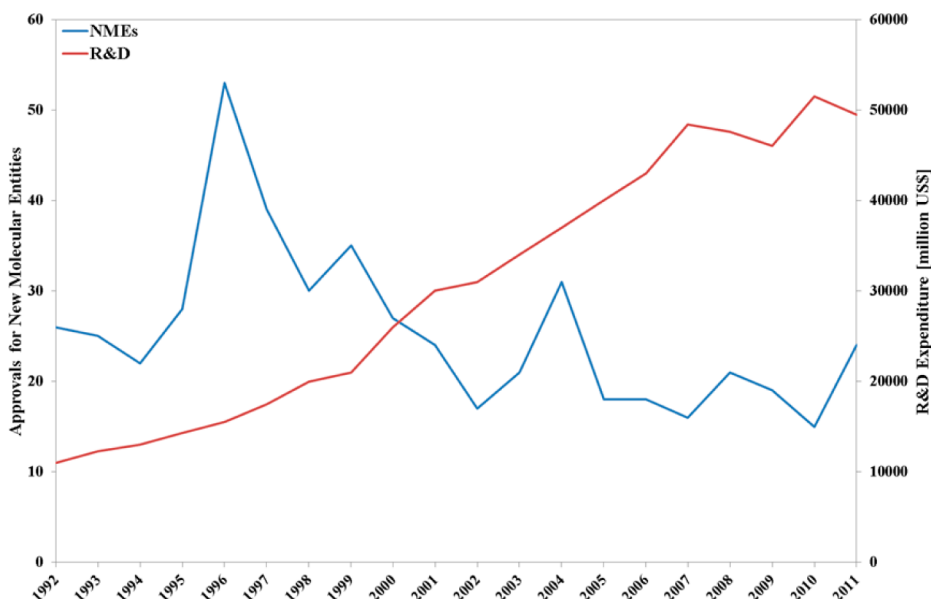
required selectivity of the tracer depends not only on the relative affinity for competing biological structures but also on the level of expression and distribution of those targets in the brain. For example, a labeled  $\text{D}_4$  receptor ligand requires a much higher selectivity over other  $\text{D}_2$ -like receptors than does a radiotracer targeting the  $\text{D}_2$  receptor, due to the much lower expression levels of the  $\text{D}_4$  receptor. Selectivity over other binding sites of at least 30-fold is recommended to obtain adequate selectivity and unambiguous results.

**Targets.** Because of the properties of the organic positron emitters and the various requirements for the final radiotracer, not all interesting CNS targets can be readily investigated by existing methods. As noted above, large molecules such as proteins or nanoparticles are not amenable to application with the generally short-lived PET nuclides due to the slower kinetics of their binding at target sites and their low permeability to the BBB. Hitherto, most useful brain PET tracers have been small molecules targeting transmembrane receptors and transporters and intracellular and extracellular enzymes of high activity.

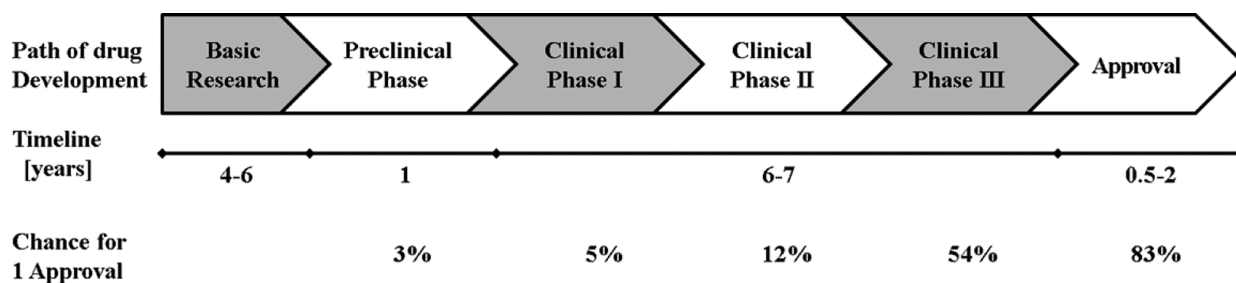
**Strategies for the Development of Radiotracers.** The development and optimization of totally new lead structures for biological targets is a time-intensive and costly endeavor. Because most radiotracers are developed in academic facilities, which usually suffer from limited resources, new tracers are typically based on lead structures derived from other natural science departments, e.g., pharmacy or biology, or are adopted from structures that were developed by pharmaceutical companies but did not proceed to clinical evaluations. This is unavoidable, since the properties required for clinically effective drugs and those for radiotracers differ substantially. However, in recent years, there has been a tremendous increase in interest in the part of pharmaceutical companies in the development of new PET tracers, e.g., Piramal Healthcare, GE Healthcare, or Avid Radiopharmaceuticals. This is true in particular for the design of new tracers visualizing beta-amyloid ( $A\beta$ ) plaques in Alzheimer's disease (AD) or mild cognitive impairment (MCI). Whereas the reference method for differential diagnosis of diverse neurodegenerative disorders is still based on histology (which is clinically, however, not helpful), the clinical differentiation between dementias (even including anatomical imaging) is not entirely robust. Consequently, there is great interest in finding diagnostic methods for the presence and distribution of  $A\beta$  plaques and neurofibrillary tangles in the brain, which are the pathologic hallmarks of AD. This interest is driven by the burgeoning prevalence of dementias and by the

search for effective interventions. A classic example of PET tracer development began with the compound Thioflavin T (6, cf. Figure 10), a dye long used for the histological staining of  $A\beta$  plaques. Because such a benzothiazole salt does not cross the BBB, a research team from the University of Pittsburgh optimized this lead structure by demethylation of the thiazole ring and derivatization of the aniline and the methyl group in 6-position, finally obtaining 2-(4'-methylaminophenyl)-6-hydroxybenzothiazole, the so-called Pittsburgh compound B (PiB, cf. Figure 10).<sup>38</sup> In the first PET study with this tracer in AD patients, tracer uptake was enriched in areas of the cerebral cortex, known from postmortem studies to contain significant amounts of  $A\beta$  plaques in dementia patients.<sup>39</sup> However, due to the short half-life of carbon-11, this tracer can be used only by PET sites with an on-site cyclotron. To circumvent this limitation and to allow a broader application of this type of tracer, an  $^{18}\text{F}$ -fluorinated derivative was developed. By means of H-to-F substitution (cf. Isotope and Analogue Tracers) of the hydrogen in ortho-position to the aniline function, [ $^{18}\text{F}$ ]-flutemetamol (cf. Figure 10) was obtained,<sup>40</sup> which shows a distribution in AD brain comparable to that of [ $^{11}\text{C}$ ]PiB.<sup>41</sup> Congo Red (cf. Figure 10) is another dye used for staining  $A\beta$  plaques, which served as an alternative lead compound for an AD PET tracer. To decrease the molecular weight and to increase the metabolic stability and lipophilicity of the Congo Red pharmacophore, derivatives of salicylic acid, e.g., chrysinamine G, and divinylbenzene compounds such as X-34, were developed.<sup>42</sup> Although these compounds showed high logD values, they failed to exhibit good brain uptake in preclinical studies. To enhance passage of the BBB, polar moieties were removed, and the molecular weight was further reduced, yielding stilbene derivatives as promising lead structures. A cooperative effort between a research group of the University of Pennsylvania and the newly founded company Avid Radiopharmaceuticals was started to further optimize this lead structure, an effort that finally resulted in the development of [ $^{18}\text{F}$ ]-florbetaben<sup>43</sup> and [ $^{18}\text{F}$ ]-florbetapir.<sup>44</sup> Hence, by adding a polyethylene moiety to the stilbene, a more appropriate lipophilicity was obtained, without loss of specificity; these tracers show good uptake in  $A\beta$  plaques as well as rapid clearance of nonspecific bound tracer. All three fluorinated tracers, [ $^{18}\text{F}$ ]-flutemetamol, [ $^{18}\text{F}$ ]-florbetaben, and [ $^{18}\text{F}$ ]-florbetapir, received FDA approval for the estimation of  $A\beta$  plaque density in the human brain by means of PET; it remains uncertain which of the three is most suitable.





**Figure 11.** Graphical representation of new molecular entities developed versus research and development (R&D) expenditure of the pharmaceutical industry during the past 20 years.<sup>46</sup>



**Figure 12.** Diagrammatic representation of the critical path initiative for drug development, showing the typical time span for each development phase and percentage chance that a drug candidate in that phase has of achieving final approval.<sup>50</sup>

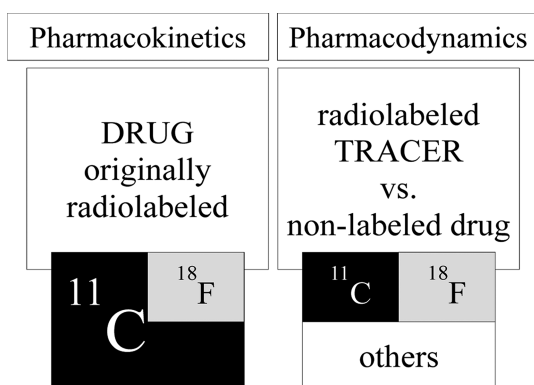
### ■ PET IN CNS DRUG DISCOVERY

Significant improvements in human longevity and health have resulted from the development of new pharmaceuticals during recent decades. During this time, the standards for research and development (R&D) of new drugs became significantly more stringent, especially through the introduction of good manufacturing practice (GMP) in the U.S. at the end of the 1930s. Later, these guidelines were expanded to improve further quality management by the introduction of good laboratory practice (GLP) and good clinical practice (GCP). Adherence to these guidelines has increased the expenditure of time and money required for the development of new pharmaceuticals, cf. Figure 11. For example, haloperidol, an antipsychotic (AP) drug for the treatment of schizophrenia, was licensed for the first time in Belgium within 2 years after its development had begun in 1957. In contrast, new pharmaceuticals reach the market nowadays after an average of about 13 years of development.<sup>45</sup> On the other hand, new methods for efficient investigation of drugs have been established; these innovations include databases for an improved identification of lead compounds, combinatorial chemistry, which drastically increases the number of compounds a chemist can synthesize per year, and high-throughput screening, which allowed for faster testing of a library of compounds. Despite these innovations, the number of new molecular entities getting approval has steadily declined over recent decades (Figure 9).

In 2004, the U.S. Food and Drug Administration (FDA) introduced a *critical path initiative* to modernize and increase the efficiency of drug development and to overcome the stagnation in the registration of new pharmaceuticals.<sup>47</sup> The critical path, as specified in that initiative, outlines the typical development route for new drugs, starting after the initial research and discovery phase but consisting of the preclinical, clinical, and approval phases, cf. Figure 12. The aim of the initiative was to implement new scientific tools within the critical path of drug development, to increase R&D efficiency, and to improve the product development. Increased R&D efficiency is desirable, since today only 1 to 2 of every 10 000 substances synthesized in the basic research phase will successfully pass all stages of the critical path and finally achieve approval. Even in the clinical phases, there is a high rate of failure, resulting in an especially high financial expenditure at this late point. For example, only 12% of the compounds recently tested in the clinical phase II were ultimately approved, even though some 40% of the total R&D expenditures to develop a new drug are already spent at this stage.<sup>48</sup> In order to ameliorate this state of affairs, molecular imaging is one of the key technologies explicitly mentioned in that initiative.<sup>49</sup> PET can support this program by giving fast access to in vivo data in the preclinical phases to verify the proof of concept or in the clinical phases to optimize the dosage and establish the efficacy of the drug.

## MEASUREMENT TECHNIQUES FOR PET IN CNS RESEARCH

Different PET techniques can be used for the determination of pharmacokinetic and pharmacodynamic properties of drug candidates during the preclinical and clinical phases of development. The choice of technique depends on the availability of validated PET tracers as well as on the properties of the drug candidate and the end point parameters of interest. Approaches can be structured into direct and indirect categories: the selection of the method naturally influences the nature of the end-point parameters to be measured. If the new drug can be labeled, then its pharmacokinetic properties can be measured via the direct method, while pharmacodynamic properties are examined using indirect methods, relative to effects on established radiotracers, cf. Figure 13.

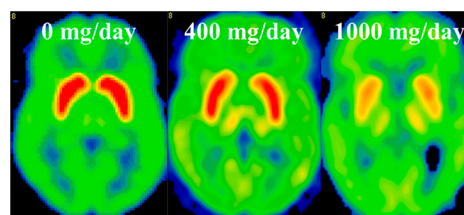


**Figure 13.** Determination of pharmacokinetics and pharmacodynamics via direct or indirect measurement. Labeling for the direct method is mainly performed using <sup>11</sup>C-carbon, but <sup>18</sup>F-fluorine may be preferred if the molecule contains fluorine. For indirect methods, the radionuclide used depends on the molecular structure of the established tracer, which usually entails <sup>11</sup>C- or <sup>18</sup>F-labeling, although radionuclides such as <sup>76</sup>Br or <sup>124</sup>I are occasionally used.

In the direct method the drug candidate itself is labeled to allow measurement of its biological parameters from the PET data. Parameters such as uptake across the BBB, distribution, reversibility of target binding, and wash out can be quantified by application of compartmental models. To avoid any changes in its pharmacology, the drug candidate must be isotopically labeled, most frequently with <sup>11</sup>C or <sup>18</sup>F (if applicable). For example, FK960 (*N*-(4-acetyl-1-piperazinyl)-*p*-fluorobenzamide) is a drug candidate for the treatment of dementia, which acts by raising acetylcholinergic levels in the brain hippocampus via somatostatin release.<sup>51</sup> It had shown promising properties in rodent and nonhuman primate models of dementia. Because FK960 showed a bell-shaped dose–response relationship, it was isotopically labeled with <sup>18</sup>F-fluorine in order to examine the basis of this relationship in vivo. Subsequently, a PET study in rhesus monkey was performed, which showed that [<sup>18</sup>F]FK960 penetrated the BBB and distributed dose dependently throughout brain. The results also suggested that this PET imaging method could be used to determine the optimal FK960 brain concentrations in humans and to objectively establish the threshold for the declining phase of the dose–response relationship.<sup>52</sup>

In contrast, the indirect method makes use of a well-known, i.e., validated, PET tracer to examine the drug candidates behavior. Here, the PET tracer is selected to reveal direct or

indirect competition with the drug candidate for the biological target of interest. In the case of receptors and transporters, this can give important information such as total density and/or availability of target receptors ( $B_{max}$ ), the effect of the drug on apparent affinity ( $K_D$ ), or the ratio of the Michaelis–Menten constants, corresponding to the ligand binding potential (BP). The indirect methods also allow determination of the dose-dependent receptor occupancy, which provides insight into the maximum dose or dosing interval of the drug candidates. For example, the striatal dopamine receptor occupancy obtained with increasing doses of the atypical antipsychotic amisulpride was determined via this indirect method using the fluorinated  $D_{2/3}$  radiotracer [<sup>18</sup>F]desmethoxyfallypride (5-(3-[<sup>18</sup>F]-fluoropropyl)-2-methoxy-*N*-[(2*S*)-1-prop-2-enylpyrrolidin-2-yl]methyl]benzamide, [<sup>18</sup>F]DMFP, cf. Figure 14.<sup>53</sup>

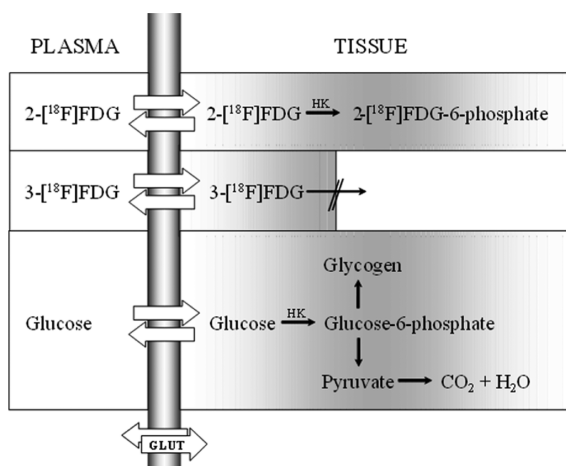


**Figure 14.** Reduced [<sup>18</sup>F]desmethoxyfallypride binding at dopamine  $D_{2/3}$  receptors due to increasing receptor occupancy level by amisulpride. The image shows tracer binding under drug-free conditions (0 mg/day) and under stable daily doses of 400 and 1000 mg of amisulpride, respectively. The normally high binding (indicated by red) of the radiotracer in striatum, where  $D_{2/3}$  receptors are most abundant, is 80% blocked at very high doses of amisulpride. The 80% threshold is considered to be relevant to the emergence of extrapyramidal side effects of AP treatment.

Detection of receptor occupancies by intra- (repeated measurement) or interindividual (control group) comparisons, using receptor ligands, provides usually reliable information about the binding properties of drugs. The receptor occupancy can be easily calculated by comparing the relative changes of binding potential according to the equation  $(BP_{control} - BP_{treatment})/BP_{control} \times 100$ . For most drugs, these investigations are sufficiently reliable to calculate a plasma level vs occupancy relationship:  $occupancy (\%) = occupancy_{max} \times C_{drug}/EC_{50} + C_{drug}$ . Sometimes, the endeavored drug effect, however, is driven by secondary modulations of endogenous transmitter concentrations. This is frequently done by the inhibition of reuptake inhibitors (e.g., selective serotonin reuptake inhibitors) or by the use complex interactions of a transmitter system (e.g., agomelatine associated dopamine release by 5-HT<sub>2C</sub> antagonism). To estimate these modulations in transmitter release, intraindividual comparisons of binding potentials in receptor PET can be used to quantify the ligand competition to endogenous transmitters. This frequently used paradigm appears to be straightforward but practically suffers from some limitations. Thus, in most cases, these changes in ligand binding fail to be congruent with results (time course and concentrations) of microdialysis measurements. This depends on ligand properties and is most likely explained by biological reactions caused by the challenge (e.g., receptor internalization). Thus, these endogenous transmitter challenge paradigms usually provide only surrogate parameters with more or less face validity. Furthermore, the effects are rather low (e.g., 10% to a max of 30%  $BP_{ND}$  decrease of low-affinity  $D_2$

receptor ligands even after intake of dopaminergic stimulants like amphetamine or methylphenidate). Whereas most of these studies were done with antagonistic ligands, recently, an emerging number of studies used agonist ligands, which show distinct binding properties at high/low activity states of receptors and might provide higher sensitivities.<sup>54</sup>

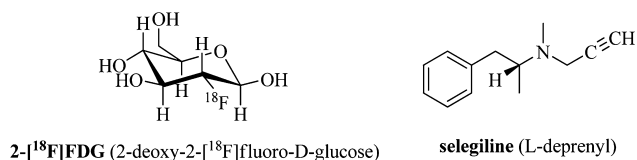
Another indirect approach can also be applied by measuring a functional activity response, such as the influence a drug candidate on brain metabolism or cerebral blood flow. There are well-established radiotracers like [<sup>15</sup>O]H<sub>2</sub>O, which is used for measuring cerebral blood flow, and glucose metabolism is measured with the analogue 2-[<sup>18</sup>F]FDG. Because of its wide use in nuclear medicine, 2-[<sup>18</sup>F]FDG is one of the most important PET tracers and is especially relevant to CNS drug development because of its well-understood metabolism and simple modeling. Glucose is transferred into living cells via the glucose transporters (GLUT1–6), where it is phosphorylated to glucose-6-phosphate and then either shunted to a glycogen pool or decomposed via anaerobic and aerobic respiration to carbon dioxide and water (Figure 15), with net production of



**Figure 15.** Metabolic pathways of glucose and 2-[<sup>18</sup>F]FDG (GLUT, glucose transporter; HK, hexokinase).

ATP. Like natural glucose, 2-[<sup>18</sup>F]FDG is a substrate for GLUT and for phosphorylation. However, the product 2-[<sup>18</sup>F]FDG-6-phosphate does not proceed further in the glycolytic pathway and is trapped in the living cell such that its accumulation with time is an indicator of the glycolysis rate of cells. In the CNS, glycolysis is normally the only source of metabolic energy.

2-[<sup>18</sup>F]FDG PET has been used in hundreds of studies with pharmacological challenge. One such study evaluated selegiline as a pharmacologic adjunct in the treatment of cocaine addiction (cf. Figure 16), aiming to reduce the euphoria caused by cocaine infusion. Earlier PET studies had demonstrated that cocaine infusions globally reduced cerebral glucose metabolism in the brain.<sup>55</sup> Consequently, the influence of the monoamine oxidase B inhibitor selegiline on the cerebrometabolic effects of



**Figure 16.** Structures of 2-[<sup>18</sup>F]FDG and selegiline.

cocaine could be evaluated using 2-[<sup>18</sup>F]FDG as a surrogate marker for local glucose consumption. Results showed that selegiline altered glucose utilization in most brain regions<sup>56</sup> and supported its use as a candidate for the treatment of cocaine addiction.

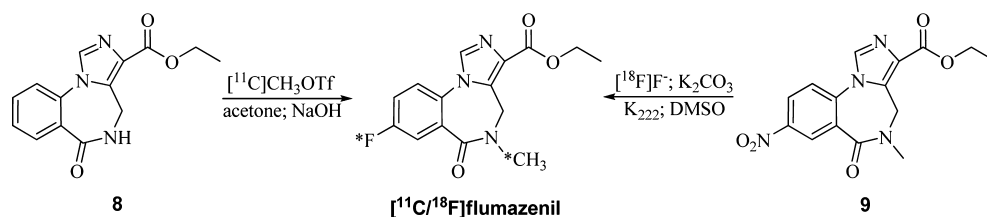
## ROLES OF PET CNS DRUG RESEARCH

PET can fulfill different roles in the development of CNS drugs. The use of PET includes, but is not limited to, four principal roles in drug research: (1) proof of biology studies, to determine if the biological target is associated with a specific disease, (2) proof of concept studies, to examine the drug candidate's biodistribution and target engagement, (3) drug dosing studies, to determine sufficient drug dosing for a clinical benefit, and (4) mechanism of action studies, to provide further insight into a drug's pharmacological behavior, such as duration of the target engagement or possible interaction with other biological targets.

**Proof of Biology.** Molecular imaging can be an important tool in the identification of a biological target, which is associated with a specific disease. Highly effective drugs exist for many diseases, but identifying new biological modes of action can substantially improve the efficiency of pharmacotherapy. The identification of new biological targets brings with it a better understanding of the pathogenesis of the disease or syndrome.

For example, migraine is a common neurovascular disorder, although a complete understanding of its pathophysiology remains elusive. Recent findings indicate that the calcitonin gene related peptide (CGRP) and its receptors (CGRP-Rs) play a crucial role in the pathogenesis and have potential for guiding development of new treatment options of migraine. The CGRP-Rs are widely distributed in the brain, in particular in the trigeminal ganglion and in the cerebellum, and are also present in the periphery on vascular muscle cells and mediate neuroinflammatory aspects of migraine. Furthermore, CGRP-R antagonists, like telcagepant or olcegepant, showed good clinical efficacy, although their primary biological target, central or peripheral CGRP-Rs, was not identified. Hence, a PET ligand for the CGRP-Rs, [<sup>11</sup>C]MK-4232 (2-[(8R)-8-(3,5-difluorophenyl)-6,8-dimethyl-10-oxo-6,9-diazaspiro[4.5]decan-9-yl]-N-[(2R)-2'-oxospiro[1,3-dihydroindene-2,3'-1H-pyrrolo-[2,3-*b*]pyridine]-5-yl]acetamide),<sup>57</sup> was undertaken to address this question.<sup>58</sup> In PET studies in healthy volunteers and in migraineurs, telcagepant in efficacious doses only results in low-to-moderate occupancies at the central CGRP-Rs, which strongly implies that an action at peripheral CGRP-Rs is at least partially involved in the treatment of migraine pain. Similar methods could help to identify substances with higher occupancies to the central receptors, which might offer additional treatment effects.

**Proof of Concept.** The aim of a proof of concept study is to examine the drug candidate's biological parameters, which may include target engagement, nonspecific binding, passage across the BBB, and metabolism. Conclusions may be then drawn concerning the suitability of the drug candidate to treat the disease of interest. As noted above, the most relevant way to obtain this information is to label the drug candidate with a positron emitter, usually <sup>11</sup>C or <sup>18</sup>F, without altering its molecular structure, since this allows direct measurement of these parameters for the actual drug. The occurrence of alkyl functional groups in many drug candidates makes labeling with <sup>11</sup>C-carbon an obvious choice. <sup>11</sup>C chemistry is well-under-



**Figure 17.** Synthesis of  $[^{11}\text{C}]$ flumazenil and  $[^{18}\text{F}]$ flumazenil, via  $^{11}\text{C}$ -methylation of the desmethyl precursor **8** and direct nucleophilic  $^{18}\text{F}$ -fluorination of the nitro-functionalized precursor **9**, respectively. The asterisks indicate the potential labeling positions.

stood, and, besides the direct irradiation products,  $[^{11}\text{C}]\text{CO}_2$  and  $[^{11}\text{C}]\text{CH}_4$ , a wide number of secondary labeling synthons are also available.<sup>59</sup> These synthons increase the versatility and applicability of  $^{11}\text{C}$ -labeling pathways, despite the short half-life of the radionuclide. Prominent examples are  $[^{11}\text{C}]\text{CH}_3\text{I}$  and  $[^{11}\text{C}]\text{CH}_3\text{OTf}$ , both of which can be used for the methylation of nitrogen, oxygen, or sulfur atoms.

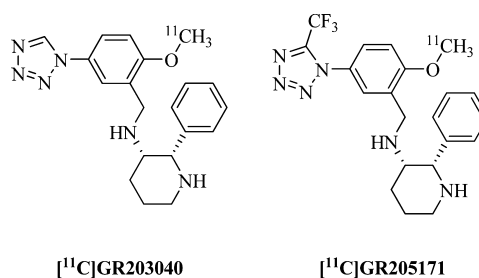
The short half-life of  $^{11}\text{C}$  remains a major limitation in the logistics of performing PET studies with a large number of patients or volunteers, since each study requires a new radiosynthesis. In comparison,  $^{18}\text{F}$ -fluorine, with a half-life of 109.7 min, is more appropriate for more complex synthetic routes and extended imaging time periods and can permit many human PET examinations from a single production batch.

The presence of fluorine in pharmaceutical lead structures, a prerequisite for isotopic labeling, has increased in recent decades, due to its favorable influence on pharmacological parameters, which include improved stability, reduced metabolism, improved affinity, and enhanced lipophilicity.<sup>60</sup> However,  $^{18}\text{F}$ -labeling strategies remain much more limited than those of  $^{11}\text{C}$ -carbon. Electrophilic  $^{18}\text{F}$ -fluorinations are possible but result in radiotracers of low specific activity. Isotopic  $^{18}\text{F}$ -labeling via nucleophilic reactions with  $^{18}\text{F}$ -fluoride generally combine high radiochemical yields with high specific activities, but this requires very harsh reaction conditions for labeling.<sup>61,62</sup> This type of radiofluorination is preferred for the labeling of aliphatic and activated aromatic moieties, and occasionally  $^{18}\text{F}$ -direct fluorination of a nonactivated aromatic position is also possible. For example, flumazenil, an established  $^{11}\text{C}$ -labeled tracer for the visualization of the benzodiazepine binding site of the GABA receptor, has also been labeled with  $^{18}\text{F}$ -fluorine, cf. Figure 17. Although the meta-position of the aromatic ring of compound **9** is not activated,  $[^{18}\text{F}]$ flumazenil could be obtained in radiochemical yields of about 15–20% by  $^{18}\text{F}$ -direct fluorination.

The development of fluorinated drug candidates amenable to  $^{18}\text{F}$ -labeling would allow a better implementation of PET in the preclinical and clinical phases. However, most drug candidates do not contain fluorine, or, if present, the fluorine is not accessible via the established  $^{18}\text{F}$ -labeling strategies; this issue severely limits the application of  $^{18}\text{F}$ -fluorine in CNS drug research.

An illustrative example for the usefulness of PET for proof of concept studies with the labeled drug candidate is provided by the evaluation of two different neurokinin 1 receptor (NK1) antagonists in preclinical studies: GR203040 ((2-methoxy-5-tetrazol-1-ylbenzyl)(2-phenylpiperidin-3-yl)amine)<sup>63</sup> and GR205171 ((2*S*,3*S*)-*N*-[[2-methoxy-5-[5-(trifluoromethyl)-tetrazol-1-yl]phenyl]methyl]-2-phenylpiperidin-3-amine).<sup>64</sup> The NK1 receptor, a subclass of the tachykinin receptor, is involved in diverse processes in the CNS, which include emotional behavior, stress, depression, anxiety, migraine,

alcohol addiction, and neurodegeneration.<sup>65</sup> Antagonists of the NK1 receptor are therefore promising compounds for the treatment of relevant diseases. Because both GR203040 and GR205171 showed a high affinity for NK1 receptors *in vitro*, they were subsequently evaluated in PET studies in monkeys using the  $^{11}\text{C}$ -labeled compounds (cf. Figure 18).<sup>66</sup> Brain



**Figure 18.** Structures of the  $^{11}\text{C}$ -labeled NK1 receptor radiotracers  $[^{11}\text{C}]\text{GR203040}$  and  $[^{11}\text{C}]\text{GR205171}$ .

uptake, distribution pattern, and binding selectivity of the tracers revealed a superior brain uptake and lower nonspecific binding for  $[^{11}\text{C}]\text{GR205171}$ , highlighting it as the preferred candidate. GR205171 was further evaluated as a drug for the treatment of postoperative nausea, motion sickness, and migraines until its termination during phase II clinical trials.<sup>67</sup>

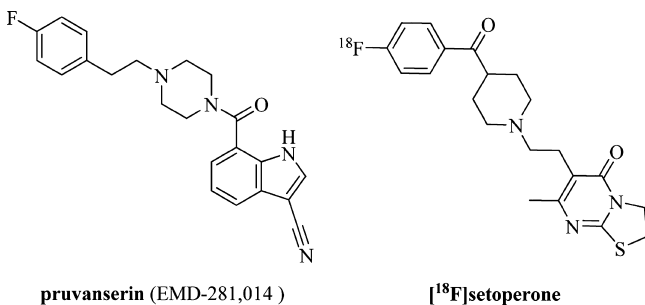
**Determination of Drug Dosing.** The determination of optimal dosing of a drug candidate represents one of the most important roles of PET in CNS research, because wrong dosing is one of the four major causes of failure in drug development.<sup>68</sup> Because PET can very often help to identify an appropriate dose range using only a small group of healthy volunteers, it can not only accelerate drug development but also may minimize the risk of adverse events and reduce the required group size for clinical trials. Neglecting the frequently performed retrospective dose/binding characterizations, dose-finding studies should usually be performed in phase I clinical trials but can sometimes contribute to phase II or even phase IV. However, phase IV dosing studies are mostly conducted as retests or in the context of a new indication for the drug. Neuroreceptor ligand dose finding by PET is now an established method, in particular for drugs targeting the dopaminergic and serotonergic systems. It even became a recommended requirement for an FDA registration of certain classes of new drugs.

In this indirect PET technique for receptor occupancy studies, a validated tracer such as  $[^{18}\text{F}]$ desmethoxyfallypride (see above) is used to label the receptor, which is then challenged with different doses of the drug candidate, to determine the drug's target engagement.<sup>69,70</sup> Because this process is well-examined, validated radiotracers are needed for the determination of receptor occupancy; most of these studies to date have been performed for drug candidates targeting

postsynaptic  $D_{2/3}$ -like neuroreceptors, given the wide availability of established radiotracers (see below).

In general, dose/binding or concentration/binding studies in humans follow two strategies: (A) an occupancy or binding range is known or can reasonably be assumed for the drug candidate. Then, the dose (or plasma concentration) can be calculated from in vivo PET data. Prominent examples of this in neuropsychiatry are the establishment of  $D_{2/3}$  receptor binding curves of antipsychotics and the characterization of presynaptic transporter blockade by antidepressants. (B) Retrospectively, a reference drug with well-known clinical efficacy should be evaluated with respect to the effective target binding ratio in order to obtain a standard occupancy/binding range for new drugs. For instance, this was carried out to provide insight into the mechanisms of antipsychotics with low-extrapyramidal motor symptoms (EPMS). In preclinical PET investigations, often performed with dedicated small animal PET instruments, a broad range of plasma concentrations can be investigated in order to evaluate the drug-binding curves and to assign the behavioral or cognitive surrogate markers of efficacy. In accordance with new strategies in drug R&D, this information becomes necessary to convey a first impression of the relevant drug-to-target binding and to detect possible efficacy thresholds or biphasic properties in its clinical action; we present an example below for the case of novel glycine transporter inhibitors.

Pruvanserin (EMD-281,014), a highly selective 5-HT<sub>2A</sub> receptor antagonist (cf. Figure 19), showed promising

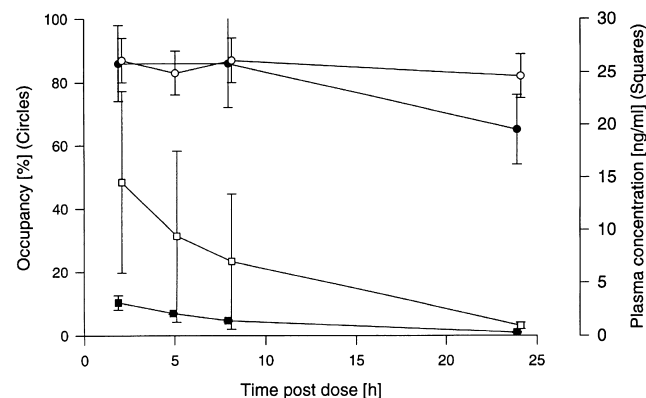


**Figure 19.** Structures of the 5-HT<sub>2A</sub> receptor antagonist pruvanserin and the <sup>18</sup>F-labeled 5-HT<sub>2A</sub> radiotracer [<sup>18</sup>F]setoperone.

therapeutic properties for the treatment of insomnia, depression, and anxiety in animal models.<sup>71,72</sup> Hence, in advance of clinical trials, a receptor occupancy study in humans was performed using the established 5-HT<sub>2A</sub> tracer [<sup>18</sup>F]setoperone.<sup>73</sup> The aims of this study were to find the oral dose of pruvanserin producing 50–90% receptor occupancy and to illuminate the relationship between dose and plasma levels. The study demonstrated that even the lowest pruvanserin dose resulted in receptor occupancies of about 63%. Furthermore, the half-life of receptor occupancy, which was approximately 24 h for the three doses used in the study, was considerably longer than the half-life in the plasma. Because of these promising results, pruvanserin was further evaluated in clinical trials extending to the end of phase II.

**Mechanism of Action of the Drug.** Mechanism of action studies aim at providing a deeper understanding of the biological interactions and properties of a drug candidate. Key mechanistic questions are whether there is a relationship between the plasma levels of a drug candidate and the target engagement and if a drug effect is caused by interaction with

one or several biological targets. In an exemplary study, Gründer et al. established the relationship of receptor occupancy and plasma levels in healthy subjects after single oral doses (10 or 20 mg) of volinanserin, a potent 5-HT<sub>2A</sub> receptor antagonist.<sup>74</sup> N-[<sup>11</sup>C]Methylpiperone was applied to determine the receptor occupancy in the frontal cortex, while plasma levels were determined using HPLC and mass spectroscopy (Figure 20). Interestingly, although a fast plasma



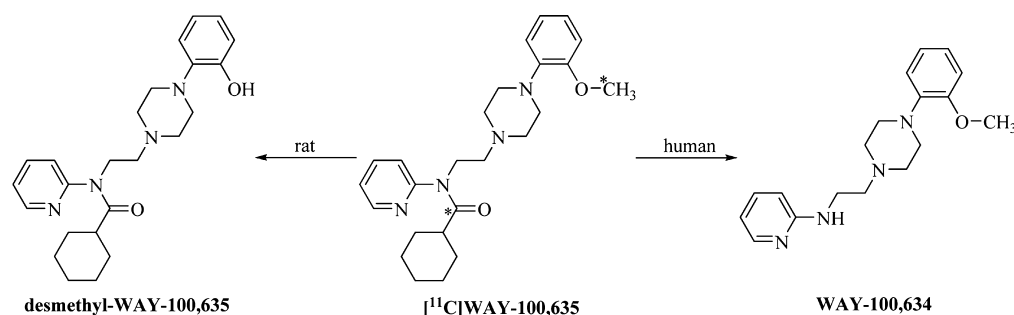
**Figure 20.** 5-HT<sub>2A</sub> receptor occupancy in the frontal cortex (mean 6 SD), expressed in percent decrease in  $BP_{ND}$  of N-[<sup>11</sup>C]-methylpiperone from baseline (left y axis, represented by circles), and plasma concentrations for 10 mg (black) and 20 mg (white) oral doses of volinanserin (right y axis, represented by squares).<sup>74</sup>

washout was observed for both doses, stable 5-HT<sub>2A</sub> receptor occupancies of about 85% occurred for 24 h for the 20 mg dose, whereas the 10 mg dose resulted in this occupancy for only 8 h, followed by a slow decrease to 70%. Hence, this study showed that there is a discrepancy between the central pharmacodynamic effects of volinanserin and its peripheral pharmacokinetics.

Another example for this approach is the systematic characterization of 5-HT<sub>2A</sub>-binding properties of antipsychotics with low EPMS properties.<sup>123–125</sup> These PET investigations detected occupancy levels exceeding 80% for a clinically effective dose regimen. Subsequent investigations were designed to prove whether this 5-HT<sub>2A</sub> antagonism is likely to perturb dopaminergic transmission. In particular, these indirect challenge investigations were frequently performed to identify interactions between two neurotransmission systems. Knowledge of these interactions is important to establish new hypothesis of drug actions.

In this context, several studies have focused on the interaction between NMDA receptor antagonism and increased dopamine transmission. Together with clinical data, these results raise the importance of new drug strategies to manipulate the NMDA receptor action for improving the outcome in the treatment of schizophrenia. Another example is an [<sup>18</sup>F]FDOPA PET study on the dopamine/norepinephrine transporter inhibition by methylphenidate (MPH), which revealed a persistent increase in dopamine synthesis capacity after short-term MPH challenge. Other investigations linked opioidergic as well as endocannabinoid systems with dopamine transmission.<sup>75</sup> The latter results may help in the development of anticraving substances and properly belong to the category of proof of biology investigations.

**Species Differences in PET Studies.** Because most of the study designs described above can be performed in both



**Figure 21.** Structure of [ $^{11}\text{C}$ ]WAY-100,635, indicating the possible labeling positions by an asterisk ([carbonyl- $^{11}\text{C}$ ]WAY-100,635 and [ $^{\text{O-methyl-}}^{11}\text{C}$ ]WAY-100,635) causing different metabolic pathways in rats and humans.

preclinical and clinical phases, it must be considered that results obtained in animal studies (mostly rodent models) need not apply perfectly to humans. For example, [ $^{\text{O-methyl-}}^{11}\text{C}$ ]WAY-100,635<sup>76</sup>, a  $^{11}\text{C}$ -labeled radiotracer of the 5-HT<sub>1A</sub> receptor antagonist WAY-100,635, showed very high affinity and selectivity in vitro. The rat in vivo studies showed the expected high uptake in the hippocampus, a 5-HT<sub>1A</sub>-rich region, and low uptake in the cerebellum, a region with low 5-HT<sub>1A</sub> expression.<sup>76</sup> However, in humans, a substantially lower ratio between receptor-rich and receptor-poor regions was measured.<sup>77</sup> An explanation for this differing in vivo behavior of [ $^{\text{O-methyl-}}^{11}\text{C}$ ]WAY-100,635 was provided by the differing metabolic pathways of the radiotracer in rats and humans. While in rats the tracer is metabolized by demethylation of the  $^{11}\text{CH}_3$ -moiety, in humans the primary metabolic pathway is through hydrolysis of the amide bond, resulting in the formation of [ $^{11}\text{C}$ ]WAY-100,634 (cf. Figure 21). This is an active metabolite with 5-HT<sub>1A</sub> binding and also high affinity for  $\alpha_1$ -adrenergic receptors in brain. Its formation in humans reduces the serotonergic signal-to-noise ratio and complicates the modeling of [ $^{\text{O-methyl-}}^{11}\text{C}$ ]WAY-100,635.<sup>78</sup> Hence, WAY-100,635 was labeled at the amide function, resulting in [carbonyl- $^{11}\text{C}$ ]WAY-100,635,<sup>79</sup> thereby circumventing the problem of the different pathways in rats and humans.<sup>80</sup>

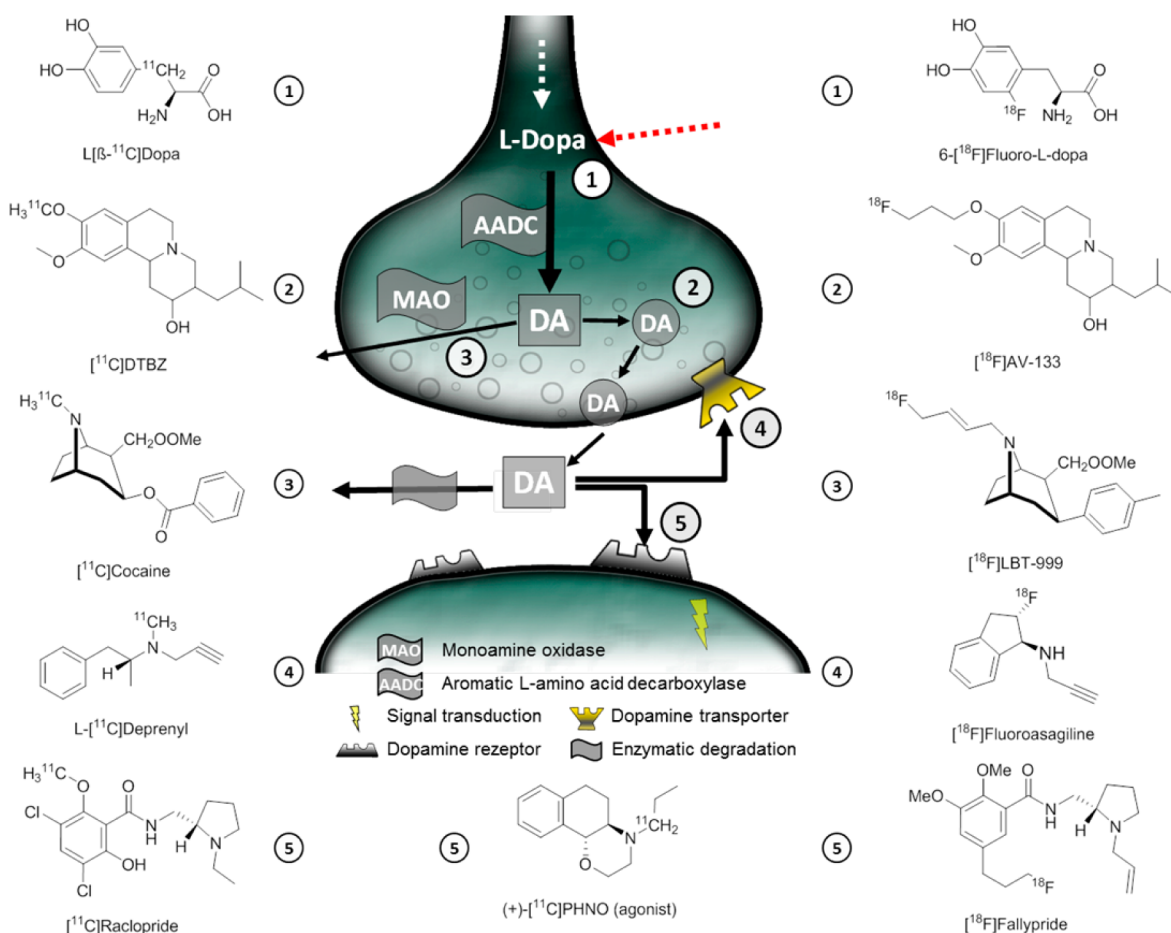
However, it is not always possible to solve problem arising from species differences by changing the labeling position. This limitation clearly demonstrates the significance of validated PET tracers in CNS drug research, which is especially important for tracers used with the indirect PET techniques, since unanticipated results might prove to be caused by an unexpected property of the drug or the tracer.

## ■ PET IN CLINICAL DRUG DEVELOPMENT USING THE EXAMPLE OF ANTIPSYCHOTICS

The major issues in drug development can be summarized as proof of biology, proof of concept, dose finding, and evaluation of drug action, as noted above. PET may impact each of these issues via different technical approaches. Whereas it is nearly self-evident that PET can help in dose finding when suitable ligands are available, reasonable applications in the fields of proof of biology and proof of concept are much more complex. Its use is generally more favorable in drug development when underlying concepts and biology are not fully clear or are insufficiently grounded. In CNS drug development, in particular for mental disorders, this is frequently precisely the situation. Historically, it might be argued that nearly all relevant treatment strategies for neuropsychiatric disorders depend on incidentally discovered drug effects. PET was not available until

the 1980s, long past the early developmental stages of antipsychotics and antidepressants. However, the potential for these old treatment strategies appears now to be exhausted, and conventional drug discovery programs have been disappointing in terms of innovative psychiatric treatment options. This is particularly true for antipsychotic drugs. Nevertheless, scientists and drug developers soon came to realize the potential impact of PET in this field. PET aids in antipsychotic drug (AP) development not only for dose-finding but also now extends to all four key aspects of drug discovery. While PET cut its teeth with highly retrospective analyses, pharmaceutical companies now make use of PET in the entire pathway of the developmental process. There is hardly another field making as much use of PET. In the next section, we focus on the prototypic process of drug development in psychiatry, emphasizing schizophrenia.

**The Dopamine System in Psychiatry.** We do not propose to review the entire literature on PET studies provided for CNS drug discovery but to focus on the dopamine system. The historical emphasis on dopamine imaging arose for three reasons. First, the dopamine system was among the first neurotransmitter systems to be linked with a neuropsychiatric disorder. Second, brain dopamine was one of the earliest targets of CNS PET studies, and, third, PET studies have had considerable impact of dopaminergic drug discovery. Thus, the case of dopamine is an illustrative example for the demands, principles, and challenges of drug discovery in psychiatry, as reflected by its historical importance in the history of drug development. Modulating dopamine transmission is an essential mechanism in the treatment of several neuropsychiatric diseases and syndromes, e.g., schizophrenia, psychotic syndromes in mood disorders, states of delirium, Tourette's syndrome, and attention-deficit hyperactivity disorder (ADHD). Furthermore, brain dopamine is highly implicated in the neuropathology of addictive disorders, obsessive-compulsive disorders (OCD), certain variants of dementias/neurodegenerative disorders, and aggressive syndromes. Early studies focused on the anatomical pathways, receptor function, and physiology of this neurotransmitter system, which has a importance disproportionate with the numerically small population of dopamine neurons. Animals studies *ex vivo* and human postmortem examinations employed histological and electrophysiological methods. The pioneering work of Dahlström and Fuxe (1964)<sup>81</sup> established the nomenclature of the catecholamine cell groups. In psychiatry, the A8-A10 neurons are of particular importance: A8, the retrorubal field projects to the amygdala, A9, the substantia nigra projects to dorsal striatal regions with mainly motor-associated functions, and A10, the ventral tegmental area



**Figure 22.** Diagrammatic representation of a striatal dopaminergic synapse, showing relevant steps of dopaminergic neurotransmission: (1) presynaptic L-Dopa uptake and decarboxylation, (2) vesicular monoamine uptake, (3) oxidative deamination of dopamine by the MAO, (4) presynaptic dopamine transporter binding, and (5) postsynaptic dopamine receptor binding; the corresponding most prominent radiotracers for visualizing the individual biological targets are also shown.

(VTA) neurons, which projects to mesocortical and mesolimbic structures including the nucleus accumbens. In the target regions, dopamine exerts its actions via two classes of receptors: D<sub>1</sub>-like receptors consist of D<sub>1</sub>/D<sub>5</sub> receptors, and D<sub>2</sub>-like receptors consist of D<sub>2</sub>/D<sub>3</sub>/D<sub>4</sub> receptors. The five subtypes have very distinct distributions and concentrations as well as differing affinity for dopamine and differing signal transduction pathways. Consequently, the effects of dopamine at the respective receptors provide some kind of functional antagonism and equilibrium between these pathways. Certain models for D<sub>1</sub> and D<sub>2</sub> receptors (cf. reviews by Seamans and Yang<sup>82</sup> as well as Durstewitz and co-workers<sup>83</sup>) suggest that liberal vs conservative information processing is modulated by the predominant activation of either D<sub>1</sub>- or D<sub>2</sub>-like receptors. Keeping in mind that APs are necessarily D<sub>2</sub>-like receptor antagonists, it appeared reasonable that disequilibrium might explain aspects of the pathophysiology in schizophrenia. PET methods are unsuited to investigating the fine structure of receptor distributions and function, due to lack of spatial and temporal resolution. However, PET has now attained spatial resolution as low as 1 mm and remains the only way to reveal neuroreceptors in the living brain.

#### PET Radiotracers for Use in Schizophrenia Research.

It is beyond the scope of this Perspective to provide a complete overview on PET ligands used in schizophrenia research. A vast number of candidates have been evaluated; however, in

practice, only some ligands are frequently used and provide the necessities of a suitable ligand as before mentioned. The translational scientist needs a palette of tracers that could image a reasonably broad range of the physiological and anatomical cornerstones of the transmitter system. Furthermore, these ligands should be able to be reliably synthesized, easily modeled, and, in some cases, subject to dynamic changes (competition paradigms).

In the dopamine system, this comprises the characterization of (1) dopamine synthesis, (2) the vesicular uptake transporter, (3) diverse receptor subtypes, (4) reuptake transporter, (5) enzymes of degradation, (6) second messenger systems, and finally, indirectly, any ligand that binds to structures that are in tight association with the dopamine system (e.g., cannabinoid system, adenosine receptors). Figure 22 shows a selection of radiotracers that are in use for clinical investigations.

**The Dopamine System in R&D.** An appreciation of the clinical/behavioral impact of the dopamine system as well as knowledge of dopamine-associated pathophysiology in mental disorders has been predominantly increased by the wisdom of hindsight. Early drug developments (in particular, the antipsychotic D<sub>2</sub> antagonists) depended initially on accidental observations and empirical evidence rather than theory. The antipsychotic profile was incidentally observed during the development of a sedative drug and was later linked to blockade of dopamine receptors (cf. the Nobel Prize in Physiology or

Medicine, 2000, awarded jointly to Arvid Carlsson, Paul Greengard, and Eric R. Kandel).<sup>84</sup> There remains a common tenet that clinically effective antipsychotic treatment necessarily depends on sufficient D<sub>2</sub> receptor blockade in excess. However, this mechanism is also responsible for the extrapyramidal motor side effects. In fact, more recently investigated antipsychotic mechanisms such as inhibition of the glycine transporter or mGluR<sub>2/3</sub> receptor agonists lack sufficient efficacy or are designed as add-on therapeutics. Pharmaceutical companies initially intended to produce D<sub>2</sub>-like receptor antagonists of higher affinity and with lesser sedative properties. Consequently, the development of agents with lower incidence of EPMS, while retaining antipsychotic efficacy, was of increasing interest; these are the so-called atypical antipsychotics, now better known as second-generation antipsychotics, SGA. The first SGAs with a so-called atypical profile of action arose from the retrospective and accidental observations/evaluation of known antipsychotics.<sup>85,86</sup> For the intentional design of SGA drugs, it was necessary to possess a deeper knowledge about dopaminergic regulation in the living brain and the binding properties of antipsychotic drugs. In the 1980s, PET was applied retrospectively in R&D and was mainly driven by academic institutions. As long as the pharmaceutical industry retained focus on specific targets such as the D<sub>2</sub> receptor, the impact of PET followed proof of biology and dose finding rather than proof of concept issues. However, these early studies laid the foundation for later drug development strategies.

**PET Studies of the Presynaptic Dopamine System.** As mentioned above, PET is unsuited to investigate the fine structure of dopamine transmission, due to its inherently low spatial and temporal resolution. Furthermore, the palette of available tracers does not capture the entire range of dopaminergic targets. Nevertheless, some key structures of the dopamine system are well-quantified and characterized by PET. Figure 22 shows a selection of the most important radiotracers, which target (mainly) postsynaptic D<sub>2/3</sub> receptors and presynaptic dopamine reuptake transporters or are substrates/ligands of aromatic amino acid decarboxylase (AADC) and monoamine oxidase A. Using such radiotracers, the pathway for dopamine synthesis, binding, reuptake, and metabolism of dopamine can be investigated, with implications for treatment strategies and for translational research informed by an understanding of pharmacological manipulations of target structures in vivo and of factors imparting vulnerability to neuropsychiatric disease. No other method is of similar significance for specifically characterizing molecular processes in the brain under in vivo conditions. Thus, the investigations described below have contributed to drug development in terms of the proof of biology approach.

**PET Studies of Dopamine Synthesis Capacity.** On the basis of early autoradiographic analysis ex vivo using tritiated L-Dopa,<sup>87</sup> the synthesis and application of [<sup>18</sup>F]FDOPA arose as one of the first developments for clinical and scientific brain PET investigations.<sup>88</sup> [<sup>18</sup>F]FDOPA remains a very important radiotracer, in spite of its complex metabolism, relatively low signal-to-noise ratio, and difficult neurophysiological interpretation (see below). Similar to the well-known and commonly used brain metabolism marker 2-[<sup>18</sup>F]FDG, and unlike most other radiotracers, [<sup>18</sup>F]FDOPA is an enzyme substrate, which is metabolically trapped in neurons terminals. [<sup>18</sup>F]FDOPA, together with its peripheral metabolite O-methyl-[<sup>18</sup>F]FDOPA, is cleared from blood to brain by the common amino acid

transporter for the L-Dopa precursor L-tyrosine as well as several other amino acids. Therefore, [<sup>18</sup>F]FDOPA-PET subjects are advised to abstain from protein consumption in the hours prior to scanning to minimize competition at the BBB amino acid transporter. In nerve cells, [<sup>18</sup>F]FDOPA (analogously to L-Dopa) is a substrate of aromatic amino acid decarboxylase (AADC), yielding 6-[<sup>18</sup>F]fluorodopamine.<sup>89</sup> This product is stored in the vesicles; its trapping can be considered irreversible in the course of 1 h PET scans. The interpretation of [<sup>18</sup>F]FDOPA scans is complicated since AADC is an enzyme of high capacity and is clearly not the rate-limiting step of dopamine synthesis. Nevertheless, Cumming and Gjedde<sup>90</sup> argued that AADC is also potentially regulated, since its activity defines the fraction of L-Dopa that will proceed to dopamine synthesis or will diffuse out of the brain. Many [<sup>18</sup>F]FDOPA-PET studies report as their end point the net blood/brain clearance (*K*), a macroparameter that can be described as the dopamine synthesis capacity. This parameter mirrors the anatomical and structural density of dopaminergic terminals (a rather stable influencing factor), but it is also subject to regulatory processes altering AADC activity under environmental or pharmacological challenges. A high dopamine synthesis capacity does not necessarily depict a high rate of dopamine transmission, but rather indicates that the system can respond with high capacity if needed, in the way an idling car engine can respond upon engagement of the (mechanical) transmission. Using standard compartment models for [<sup>18</sup>F]FDOPA analysis, the rate of AADC activity (*k*<sub>3</sub>) can be calculated in striatum, which predicts the capacity for dopamine synthesis from endogenous brain L-Dopa. Notwithstanding its complexity, [<sup>18</sup>F]FDOPA-PET has yielded a vast amount of clinically and scientifically important findings. In neurology, [<sup>18</sup>F]FDOPA served as an important tool to detect and monitor pathologies in the nigrostriatal dopamine system, in particular, for the diagnosis of Parkinson's disease.<sup>91,92</sup>

[<sup>18</sup>F]FDOPA-PET is no standard routine in neurology, in part due to the availability of cheaper SPECT/PET ligands for presynaptic dopamine transporters. With respect to basic investigations of mental diseases, however, its relevance continues to increase. An early [<sup>18</sup>F]FDOPA-PET study showed a 10–20% increase in dopamine synthesis capacity in schizophrenia. This finding has been replicated many times and is in some studies associated with the extent of psychotic symptoms.<sup>93</sup> However, due to the modest test–retest reliability and the interindividual variance of [<sup>18</sup>F]FDOPA-PET, group-separating differences have not been revealed in studies of schizophrenia. This could be improved by the implementation of a more sophisticated analytical approach. Using PET recordings lasting several hours and an extended compartmental model affords the estimation of the rate constant for turnover of 6-[<sup>18</sup>F]fluorodopamine formed in brain (*k*<sub>loss</sub> [min<sup>-1</sup>]); it could be observed that trapping conditions were no more stable when acquiring the dynamic PET scans for much longer than 1 h (e.g., 4 h).<sup>94,95</sup>

6-[<sup>18</sup>F]Fluorodopamine is protected against degradation when entrapped in synaptic vesicles, but it is vulnerable to deamination by MAO-A when passing through the cytosol, either as a newly synthesized molecule or upon vesicular release and subsequent reuptake. According to this model, *k*<sub>loss</sub> is a surrogate parameter for the presynaptic dopamine turnover occurring during a prolonged [<sup>18</sup>F]FDOPA PET scan. The estimation of *k*<sub>loss</sub> is, however, complicated, entailing the uses of some procedure for subtracting the contribution of O-methyl-



[ $^{18}\text{F}$ ]FDOPA to the brain signal to isolate the radioactivity due to [ $^{18}\text{F}$ ]FDOPA, [ $^{18}\text{F}$ ]fluorodopamine, and deaminated metabolites.<sup>96</sup> On the basis of tracer concentrations measured in arterial plasma samples during a 2 h PET scan,  $k_{\text{loss}}$  can be calculated for striatal regions and the midbrain. Although  $k_{\text{loss}}$  does not have a simple physiological interpretation, it is a depiction of the washout or turnover of tracer dopamine and has yielded astonishing results, such as in a clinical PET study of schizophrenia. A 10–20% increase in synthesis capacity was detected, as reported in the meta-analysis, while the dopamine turnover rate was doubled, which can be considered one of the most pronounced neurobiological findings in schizophrenia research.<sup>97</sup> This finding implies what might be described as churning, which might subserve the positive symptoms of psychosis but is also in accordance with theories to explain the neurocognitive impairments in schizophrenia.

Whereas the impact of APs on the postsynaptic sites has been a focus of drug development, the consequences of AP action on presynaptic regulation of dopamine metabolism has been neglected. [ $^{18}\text{F}$ ]FDOPA-PET studies, however, have shown that APs do not simply block signaling at postsynaptic  $\text{D}_{2/3}$  sites but cause initial upregulations and later a profound downregulation of dopamine synthesis capacity or AADC activity.<sup>98–100</sup> These investigations focus on acute modulation and finally on the normalization of elevated dopamine synthesis capacity, one of the oldest PET findings in schizophrenia. Whereas in pigs an acute AP challenge increased  $K$ , this effect was observed in humans after treatment for 3 days.<sup>99,101</sup> A significant decrease of  $k_3$  was reported by Gründer and co-workers after 4 weeks of ongoing haloperidol treatment according to clinical needs of acutely psychotic schizophrenia patients.<sup>98</sup> Both effects on dopamine synthesis and turnover, the initial increase as well as the later decrease, were of substantial magnitude (approximately 20–30%). Thus, prolonged antipsychotic treatment normalized the pathologically upregulated dopamine system in the basal ganglia. This effect correlated with the clinical outcome for negative symptoms, in spite of rather low group sizes. The PET data support very early investigations in the cerebrospinal fluid (CSF) of patients suffering from schizophrenia, which also gave a similar impression of dopaminergic regulation in the course of AP treatment. One such CSF study showed, in fact, just such an association between the metabolite levels and the later treatment response.<sup>102</sup>

Whereas these investigations are designed to better understand the mechanisms of the pathophysiology in well-diagnosed states of schizophrenia, including the respective treatment effects, [ $^{18}\text{F}$ ]FDOPA PET was also recently used to provide evidence for the stratification of patients in the prodromal state.<sup>103,104</sup> The clinical management of prodromal patients suffering from attenuated symptoms of psychosis or only brief periods of positive symptoms, without meeting criteria for diagnosis of schizophrenia, is a current focus of discussion in psychiatry. While we understand the importance of timely initiation of AP treatment in order to improve the clinical outcome, the substantial side effects of APs are problematic in subjects for whom conversion to psychosis is uncertain. The conventional clinical tools are insufficient to define a risk threshold above which treatment is more beneficial than harmful, because only a minority of patients with prodromal states convert to manifest chronic schizophrenia. Several [ $^{18}\text{F}$ ]FDOPA-PET studies could, in fact, show that increased dopamine synthesis capacity is present in groups of prodromal

patients and that the extent of the increase is predictive of conversion.<sup>105</sup>

Although PET seems unlikely to become a standard tool for diagnosis of mental disorders (with the possible exception of Alzheimer's dementia), findings in prodromal patients might help to stratify patients with respect to their clinical risks and treatment options. In the context of drug development, these data are an important contribution to the proof of biology approach; nevertheless, such results remain somewhat orphaned with respect to target development and are not yet linked to further proof of concept investigations. Nevertheless, it might prove to be crucial for treatment to reestablish a normal state of dopaminergic transmission. PET is the only tool to observe these drug effects directly in patients.

**PET Studies of Dopamine Receptors.** Compared to [ $^{18}\text{F}$ ]FDOPA, labeled  $\text{D}_{2/3}$  receptor ligands are much more integrated in the process of drug R&D. This is due to the strict association between  $\text{D}_{2/3}$  antagonism and antipsychotic effects. The intention of a number of PET investigations was to retrospectively find biological markers of the disease as well as to optimize dosing and characterize the mode of action. Early PET studies of dopamine receptors used rather nonspecific radiotracers of moderate affinity, thus revealing only the abundant receptors in striatum. Data of several investigations were equivocal; overall, there is evidence for a 10–15% increase in striatal  $\text{D}_{2/3}$  receptor availability in patients with schizophrenia.<sup>106</sup> Concerning extrastriatal regions, some studies showed decreased  $\text{D}_{2/3}$  receptor binding, whereas others suggested an increase.<sup>107–110</sup> These incongruent results may be due to group differences in duration of disease, drug-free interval, and predominant symptomatology. Importantly, the results suggest that any increase in receptor availability is less related to symptom severity but seems highest at the beginning of the pathology. Thus, increased striatal  $\text{D}_{2/3}$  receptor availability in the striatum may well occur in prodromal states. Taken together, postsynaptic  $\text{D}_{2/3}$  receptor imaging has not proven to be as informative as [ $^{18}\text{F}$ ]FDOPA with regard to the mechanisms of psychosis.

Interestingly, receptor imaging has had considerable impact on drug dosing and has yielded knowledge about drug action. Besides characterizing the target structure of APs, an early application was to define the clinically most appropriate concentration of APs, which has long been an issue in psychiatry. After discovering the crucial mechanism of  $\text{D}_{2/3}$  antagonism, researchers have endeavored to develop substances of very high affinity and comparatively high selectivity for  $\text{D}_{2/3}$  receptors. Well-known agents like haloperidol, benperidol, or fluphenazine were launched and remain in clinical use. Some decades ago, it was believed that very high occupancies at  $\text{D}_{2/3}$  receptors were desirable, albeit with the risk of evoking side effects. For a time, EPMS were regarded to be inseparable from antipsychotic efficacy.<sup>111</sup>

In the late 1980s, radiolabeled spiperone derivatives were introduced (e.g., [ $^{76}\text{Br}$ ]bromospiperone;<sup>112</sup> Cambon et al.<sup>113</sup>) for the detection of  $\text{D}_2$ -like receptors in the brain of patients under antipsychotic medication. By the use of graphical analytical methods, under approximately equilibrium conditions, the specific binding could be estimated.<sup>114</sup> Receptor availability in vivo depends on the target receptor concentration, the ligand's affinity, and upon competition from endogenous and exogenous ligands. Consequently, AP-evoked reductions in BP depict the concentration of the drug in the brain. Intraindividual (baseline vs AP-challenge or AP-treated vs

drug-withdrawal) or intergroup comparisons (untreated vs treated subjects) then enabled the calculation of CNS receptor occupancies. Initial PET investigations in large groups of naturalistically treated patients with schizophrenia demonstrated a broad range of  $D_{2/3}$  receptor occupancies, e.g., Baron et al. showed 5–72% occupancy.<sup>115</sup> Unfortunately, these early investigations suffered from highly diverse disease spectra (schizophrenia, dementia, alcohol hallucinations, etc.), non-selective radiotracers, and missing drug plasma concentrations.

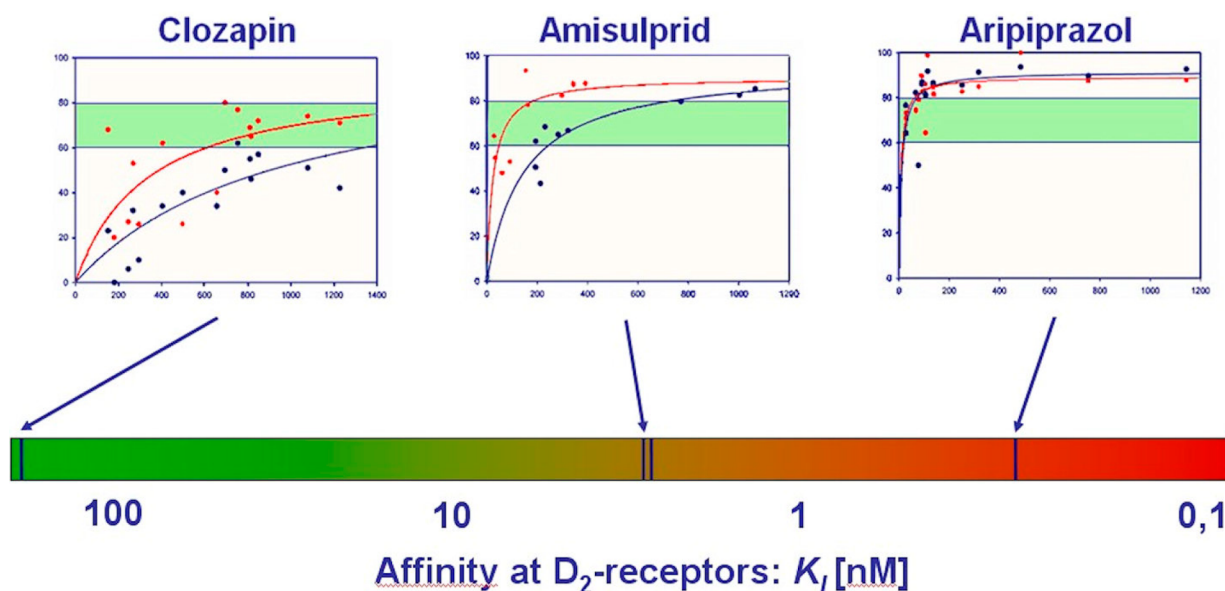
A very important improvement was the radiolabeling of substituted benzamides. The most important of these remains [<sup>11</sup>C]raclopride, which possesses moderate affinity and high selectivity at  $D_{2/3}$  receptors.<sup>116</sup> Using this radiotracer, Farde and co-workers<sup>117</sup> as well as Nordström and co-workers<sup>118</sup> published some of the most influential clinical PET studies. Patients suffering from schizophrenia and receiving effective antipsychotic treatment showed 65–85%  $D_{2/3}$  receptor occupancy in the striatum (extrastriatal receptors can scarcely be detected with [<sup>11</sup>C]raclopride). Exceeding the threshold of 80% occupancy dramatically increases the risk for EPMS such as dystonia, akathisia, and parkinsonism.<sup>119</sup> Thus, there appeared to be a range of receptor occupancy providing clinical efficacy at least against so-called positive symptoms, with lesser risk of EPMS. Discovery of this 65–80% range caused a tremendous rethinking of AP treatment regimens and gave further inspiration to later drug development. In a patient sample of Farde and co-workers, most of those agents causing lesser or no EPMS at their clinically effective daily dose were described as atypical, in contrast to typicals such as haloperidol.<sup>120</sup> In particular, clozapine and thioridazine were unlikely to EPMS. Clozapine showed clinically effective striatal receptor occupancies in a range of only 38 to 63%, i.e., less than the 65% threshold. Thioridazine, in contrast, showed comparatively high occupancies of 74–81%.

R&D focused on attempts to produce effective APs with less EPMS. For development of suitable agents, it was necessary to understand whether the absence of EPMS is simply a question of correct dosage or depends on additional (non-dopaminergic) properties of the drug. Investigations addressed these questions by dual-tracer investigations, entailing high-affinity radiotracers, along with improved analytical approaches. Early studies of non- $D_{2/3}$  receptor binding showed antagonism at M1-cholinergic receptors to be an effective mechanism to reduce EPMS.<sup>121</sup> Both thioridazine and clozapine (which were first described to exert atypical properties) show this anticholinergic receptor profile which, unfortunately, causes its own adverse effects. On the basis of the work of Meltzer and co-workers,<sup>122</sup> antagonism at the 5-HT<sub>2A</sub> receptor was also detected for some APs. Dual-tracer PET studies using standard 5-HT<sub>2A</sub> as well as  $D_2$  receptor radiotracers showed in fact a comparably high relative occupancy at the former target for a variety of second-generation APs at doses producing the before above-mentioned 65–80%  $D_{2/3}$  receptor therapeutic window. The extent of 5-HT<sub>2A</sub> occupancy was shown to consistently exceed 80% for risperidone, olanzapine, and ziprasidone in the clinically effective dose range.<sup>123–125</sup> The dual-tracer approach remains invaluable for characterizing new drugs.

Thus, PET studies have taught drug developers to design  $D_2$  antagonists that should show only a modest slope of dose/binding curves for striatal  $D_2$  receptors and/or provide a extensive 5HT<sub>2A</sub> binding at doses evoking 65–80%  $D_{2/3}$  occupancy. For example, this approach served for evaluation of the recently launched AP lurasidone: Nakazawa and co-

workers showed that this compound attained lesser 5-HT<sub>2A</sub> occupancy than did olanzapine, as revealed with the highly selective radiotracer [<sup>11</sup>C]volinanserin.<sup>126</sup> Clinical dose findings for this compound was also performed with [<sup>11</sup>C]raclopride PET, revealing that more than 40 mg is necessary to provide effective  $D_{2/3}$  receptor occupancies.<sup>127</sup> Another PET investigation of lurasidone in a large group of schizophrenia patients, however, showed that daily doses only modestly correlate with receptor occupancies, which were, however, predicted by plasma levels of the compound, along with its active metabolites.<sup>128</sup> It is well-known in therapeutic drug monitoring (TDM) that daily doses may fail to reliably predict plasma levels, clinical response, or side effects.<sup>129</sup> PET investigations, however, also uncovered that some agents, notably the AP quetiapine, fail to even reveal plasma concentration vs occupancy relationships, which calls into question the validity of simple daily dose suggestions for this compound.<sup>130,131</sup> For lurasidone, however, PET studies revealed that TDM is highly suitable. The case of lurasidone also made evident that drug characterization is not always completed during drug development. Despite the existence of suitable ligands, interesting questions regarding lurasidone's clinical binding properties at 5-HT<sub>1A</sub> as well as the promising target 5-HT<sub>7</sub> remain unanswered, although in vitro affinity predicts occupancy at these sites.<sup>132,133</sup> Emerging knowledge about the entire in vivo properties at central nervous receptors, however, is necessary in order to better predict clinical effects in the future.

A suitable AP efficacy-to-EPMS ratio does not necessarily depend on multireceptor binding profiles. For example, sulpiride, a substituted benzamide, was early considered to possess atypical action and was used as an antidepressant.<sup>134</sup> The PET study noted above revealed it to have 78%  $D_{2/3}$  receptor occupancy.<sup>120</sup> Amisulpride, another substituted benzamide, which assumed a greater popularity, was much better evaluated by  $D_{2/3}$  receptor PET and the use of high-affinity ligands.<sup>135,136</sup> These investigations were of notable interest since amisulpride shares atypical properties but, paradoxically, acts only at  $D_{2/3}$  receptors; thus, it does not depend on any 5-HT<sub>2A</sub> or M<sub>1</sub> antagonism (possible 5-HT<sub>7</sub> antagonism remains to be established<sup>137</sup>). PET studies revealed that two different mechanisms might contribute to this property. Mainly based on the moderate affinity of amisulpride at the target receptors, the striatal plasma concentration vs occupancy curve in the striatum was less steep in comparison to that of high-affinity drugs.<sup>136</sup> When including data from high-affinity ligands, it became apparent that the corresponding curves in striatal and extrastriatal curves were not identical: in extrastriatal regions, the occupancy was very high at lower doses when striatal occupancies were still rather low. These so-called preferential binding properties<sup>138</sup> were and are still a matter of controversy discussion, since some authors claim this effect to be an experimental artifact, attributable to partial volume effects or other factors such as specific binding in the reference region. Nevertheless, several different research groups reported comparable extrastriatal binding properties for many second-generation antipsychotics.<sup>135,138,139</sup> In particular for amisulpride, ziprasidone, quetiapine, and clozapine, profound occupancy differences between the regions were detected. The highest differences were found for clozapine, which was early found to bind only moderately to  $D_{2/3}$  sites in the striatum.<sup>140</sup> Nevertheless, it showed much higher (>20%) occupancies in extrastriatal regions (i.e., thalamus and cortex). Although failing to reach the crucial 65% threshold in striatum, this threshold



**Figure 23.** Association between steepness of  $D_2$  receptor binding characteristics as well as preferential extrastratial binding with respect to the drug's affinity at  $D_2$  receptors. Occupancy curves for striatum (blue) and temporal cortex (red) are illustrated for three antipsychotics: clozapine<sup>140</sup> (low affinity), amisulpride<sup>136</sup> (moderate affinity), and aripiprazole<sup>142</sup> (high affinity) using [<sup>18</sup>F]fallypride. The green bar depicts the desired 60–80% occupancy range.

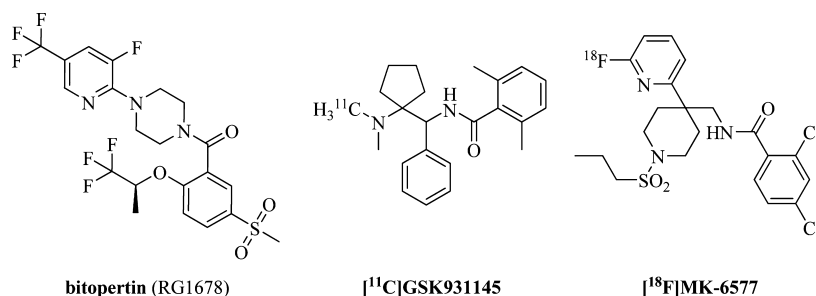
was readily attained in the temporal cortex and the medial thalamus. Similar results were obtained for quetiapine, which may lead to questioning the unique importance of striatal occupancy for AP action. Using the high-affinity ligand [<sup>18</sup>F]fallypride,<sup>141</sup> a broad sample of second-generation antipsychotics have been investigated using comparable analytical techniques. Figure 23 juxtaposes the occupancy curves with the agent's affinity at the  $D_2$  receptor. Apparently, the preferential extrastratial binding effect is associated with lower affinities, suggesting that it might arise from regional differences in competition from endogenous dopamine. In association with the naturally less steep increase of receptor binding for low-affinity ligands, a moderate-to-low affinity at  $D_{2/3}$  sites appears to be a relevant factor for atypicality, even in the absence of additional receptor binding properties. PET studies show that moderate affinity APs attain the 65–80% occupancy window for a broad range of plasma concentrations, whereas high-affinity agents usually provide only a narrow range, difficult to attain in individual patients.

Meanwhile, another mechanism to avoid EPMS was developed; aripiprazole is an antipsychotic with partial agonist properties approximately 30% that of dopamine at the  $D_{2/3}$  receptor.<sup>143</sup> In this case, AP efficacy is obtained at nearly complete receptor occupancy. Again, PET helped to provide important information about reasonable drug concentrations for aripiprazole.<sup>142</sup> Figure 23 displays the steep course of the occupancy curve, such that concentrations exceeding 100 ng/mL are very likely to cause more than 80% occupancy. This is nearly guaranteed to occur at daily doses of 15 mg or more. But daily doses below 10 mg generally achieve only low occupancies. These conclusions based on PET data are in full accordance with the results of clinical trials.<sup>144</sup> Another important issue of this study is that striatal as well as extrastratial curves are fully congruent in their course, providing evidence that preferential extrastratial binding for many other APs are not in fact due to some analytical artifact. The absence of this effect in aripiprazole and its all-or-none binding

properties can be explained by its high affinity at the  $D_2$  receptor, which exceeds that for every other second-generation antipsychotic in clinical use.

Another important question posed in PET investigations concerns the possible correlation between occupancy levels and clinical treatment effects. Because of necessarily low group sizes in PET studies, clarification of this question is generally limited by insufficient statistical power. In fact, a highly esteemed work of Agid and co-workers mentioned positive associations between clinical improvement in positive symptoms and  $D_{2/3}$  receptor occupancy in striatal but not extrastratial regions.<sup>145</sup> However, the majority of PET occupancy investigations do not mention such associations. Given that most research groups perform clinical characterization of psychopathology and treatment effects, the absence of sufficient replication studies questions this association. In general, PET investigations rather suggest a threshold effect in accordance to the pioneering investigations of Farde and co-workers, noted above.<sup>120</sup>

Most of the recent PET investigations in the field of AP development focused on dose finding as well as the optimization of antipsychotic vs side effect ratios. However, two problems in AP treatment remain unsolved. First, APs are effective only against productive positive symptoms, whereas cognitive as well as negative impairments are only modestly responsive to these compounds. The second and even more critical point is the fact that at least 40% of patients suffering from schizophrenia do not sufficiently respond to antipsychotics, even with respect to positive symptoms. Several decades on, clozapine remains the only tool for treatment resistance, with the penalty of the side effects and complications that are associated with its application. Unfortunately, many patients also fail to respond to clozapine. Until now, little has been known about the factors responsible for treatment resistance. Nevertheless, only few investigations have focused on this phenomenon. PET investigations (in particular, using presynaptic radiotracers as well as multitracer characterizations) would be very helpful in the future for the stratification of



**Figure 24.** Structures of bitopertin and the  $^{11}\text{C}$ - and  $^{18}\text{F}$ -labeled PET tracers  $[^{11}\text{C}]$ GSK931145 and  $[^{18}\text{F}]$ MK-6577 for visualization of GlyT1.

treatment-resistant patients and could inform new strategies to treat these subjects.

Returning to our theme of the principles of R&D,  $D_{2/3}$  receptor PET could highly contribute to dose finding and the proof of mechanism; a major problem has been that these investigations were mainly done retrospectively, i.e., at times after the drug was already launched. A much earlier integration in R&D is demanded to widen its relevance also for proof of concept investigations. This early integration has been attempted in the development of drugs that focus on the glutamate system rather than on the dopamine transmission.

**PET Studies on the Glutamate System in Schizophrenia.** The improvement of negative symptoms as well as cognitive impairments is a focus of recent R&D. The original dopamine hypothesis of schizophrenia was soon expanded by hypotheses that might explain the excess of dopamine transmission in the limbic system.<sup>146</sup> On the basis of functional (e.g., fMRI, CBF, and 2- $[^{18}\text{F}]$ FDG PET) studies<sup>147</sup> in schizophrenia, it was proposed that prefrontal hypofunction might cause a disbalanced cortico–striatal feedback, in turn causing impaired mesocortical but increased mesolimbic dopamine transmission. The latter mechanism might contribute to positive symptoms, whereas the downregulated mesocortical transmission might further impair cognitive functions and activity.<sup>146</sup> This hypothesis is supported by the good face-validity of experimental psychoses caused by glutamatergic NMDA receptor antagonists neglecting the critical construct validity that psychoses caused by these agents cannot be reversed by  $D_{2/3}$  antagonists.<sup>148,149</sup> Although microanatomical investigations have failed to report consistent deficiencies in the NMDA receptor system of patients, a series of clinical investigations in the last decades supported the influence of the glutamate system in the pathophysiology of schizophrenia. There is a tight interplay of dopamine signaling and NMDA-mediated signal transduction directly at cortical pyramidal cells or mediated by GABAergic interneurons. Excessive direct activation of the NMDA receptor, however, causes neurotoxic effects. Modulation of NMDA receptors is mediated by glycine, which is an essential coagonist for the opening of the NMDA receptor channel. Glycine levels at the NMDA receptor are regulated via the glycine transporter type-1 (GlyT1). Thus, antagonists of this transporter should result in an activation of NMDA receptors; GlyT1 is thus a promising biological target for the treatment of schizophrenia. In fact, pioneering work of Heresco-Levy beginning in the 1980s showed marked improvements in the negative symptoms (approximately 15%) and cognition in schizophrenia by administration of glycine or the agonists *D*-cycloserine or *D*-alanine.<sup>150</sup> The clinical use of this approach, however, was clearly limited by the poor blood–brain permeability of these agents. The next step was then to

increase glycine in the CNS by inhibition of GlyT. This approach was very early accompanied by the development of suitable GlyT1 radiotracers  $[^{11}\text{C}]$ GSK931145<sup>151</sup> and  $[^{18}\text{F}]$ MK-6577,<sup>152</sup> which have potential for occupancy investigations, cf. Figure 24. Both tracers showed high brain uptake and a displaceable distribution pattern that matched the known GlyT1 expression in brain. Very recent animal data yielded the astonishing result that the glycine transporter need only be blocked to a limited extent, whereas higher occupancies cause an inverse effect. For the GlyT1-antagonist bitopertin,<sup>153</sup> for example, the range of effective GlyT1 binding was between 10 and 30%. Eddins and co-workers reported that this range of occupancies evoked significant improvement in a model of schizophrenia negative symptoms, scopolamine-induced impairments of object retrieval.<sup>154</sup>

Concerning the human GlyT1 transporter, PET has already been used to establish reliable concentration–occupancy relationships.<sup>155</sup> Although some phase III studies had difficulties in showing the effectiveness of the new compounds against the primary end-point parameters, the development of glycine transporter antagonists is an encouraging example of the implementation of PET in R&D of neuropsychiatric disorders.

Finally, second messenger systems are a focus on new strategies in R&D. In particular, the degradation of cyclic adenosine/guanosine monophosphate (cAMP/cGMP) by phosphodiesterase (PDE) isoenzymes is an emerging topic in psychiatry in recent years. Within this superfamily of 11 isoenzyme families consisting of even more splice variants, in particular, PDE2a, PDE4, PDE5, and PDE10 are of interest for pharmacological interventions. Blockade of PDE may result in increased cAMP and cGMP levels, an effect that is comparable to the result of  $D_2$  receptor blockade. However, not only has the theoretical background encouraged further target development but also disturbed PDE regulation in mental disorders and effects on animal models of cognition and antipsychotic effects have been reported.<sup>156,157</sup> In order to provide suitable tools for in vivo target evaluation and candidate characterization, PET could be helpful, as mentioned before. In fact, for the development of PDE10 antagonists, the development of PDE10 ligands parallels the early phases of candidate evaluation. The evaluation of the specific PDE10 ligand  $[^{18}\text{F}]$ JNJ42259152 (2-((4-(1-(2- $[^{18}\text{F}]$ fluoroethyl)-4-(pyridin-4-yl)-1*H*-pyrazol-3-yl)phenoxy)methyl)-3,5-dimethylpyridine)<sup>158</sup> was originally warranted by the therapeutic impact of this second messenger system.<sup>159</sup>

Looking back at the history and status of R&D focusing on APs, the impact of PET as well as a shift in its use over the years becomes apparent. Initially, PET focused on the empirically defined target of well-established agents. Later,

available drugs were better characterized by PET not only in their therapeutic effects but also in their dose/effect relationship. Today, PET is an accepted tool for drug development. Although frequently not implemented in very early phases of R&D, it contributes to identifying targets and rational dose finding.

## ■ STRATEGIES, NEW PROGRESS, AND NEW CHALLENGES

Because most of PET tracer development is still done in academic facilities, which often lack the capacity for complete lead optimization, the development of radiotracers for new biological targets usually depends on the availability of suitable lead compounds. Hence, while, for some CNS systems, a broader array of suitable radiotracers exists (e.g., dopamine, serotonin), for others they are still missing (NMDA, GABA). Furthermore, the development of new radiotracers is also influenced by the current paradigms and research hypotheses of those science departments, which finally apply them in PET studies, e.g., nuclear medicine, psychiatry, and others. Thus, the crucial question of whether to improve the properties of radiotracers for known targets or to develop radiotracers for new biological targets cannot be answered by the radiopharmaceutical chemist alone.

However, there is no doubt that molecular imaging with PET tracers can have a significant, even decisive, impact on R&D of new drug candidates. While there remain some problems related to its more widespread use, there has been great progress.

**Chemical Aspects. Isotopic Tracers I.** The resynthesis of a certain drug candidate yielding the identical yet  $^{11}\text{C}$ -containing molecule, in general, demands an appropriate, i.e., efficient and fast,  $^{11}\text{C}$ -radiopharmaceutical chemistry. Although impressive progress has been made here over the last 2 decades, important new developments are ongoing. Issues still awaiting substantial progress include finding a useable route to introduce  $^{11}\text{C}$  directly into the structure of an aromatic ring.<sup>160</sup>

**Isotopic Tracers II.** Because of the logistic advantages of  $^{18}\text{F}$  vs  $^{11}\text{C}$ ,  $^{18}\text{F}$ -labeled isotopic tracers would be an ideal option for cases when the drug candidate already contains fluorine in its structure. Interestingly, there has been a growing tendency toward a more frequent use of fluorine as an inherent constituent of drug candidates. Nevertheless, in many cases, the introduction of  $^{18}\text{F}$  into the labeling precursor requires sophisticated radiopharmaceutical chemistry. A key challenge here concerns nucleophilic substitutions in weakly activated aromatic rings, which was recently addressed by Ritter and co-workers.<sup>161</sup>

**Tracers as Lead Structures.** The systematic development of  $^{11}\text{C}$ - and  $^{18}\text{F}$ -labeled tracers for basic neuroscience research as well as for patient diagnoses has provided an impressive number of high-affinity and highly specific PET tracers targeting a variety of brain neuroreceptors and their many subtypes. These well-studied tracers can serve as a database for the development of new drug candidates, constituting a drug surrogate, at a given target. The many tracers already available to characterize dopaminergic neurotransmission present an excellent example. Radiopharmaceutical chemistry research strives to develop adequate radiotracers for a broader range of neurotransmitter systems. In parallel,  $^{11}\text{C}$ - and  $^{18}\text{F}$ -labeled tracers have become available for other targets in the brain, such as amyloid plaques or neurofibrillary tangles. Hence, drug

candidates aiming at newer biological targets may easily benefit from the existing database of diagnostic PET tracers.

**Clinical Aspects.** The implementation of PET in clinical drug development is highly advanced with respect to dose-finding studies. Whereas this indication was previously performed rather retrospectively, it now frequently accompanies the R&D plan. Furthermore, the proof of biology, i.e., studies investigating the mechanisms of diseases, is now an emerging field in drug development, which rather indirectly supports R&D strategies. However, PET still needs to be better integrated in the developmental process at an early phase, using a choice of radiotracers well-suited to the target or treatment effect. Another important demand is the establishment of patient stratification studies using PET, aiming to identify biologically defined subgroups showing different pathophysiology or treatment response. Such strategies are expensive, considering the costs of large-scale PET studies in patients, and will depend on a close collaboration and interrelation among clinicians, chemists, and pharmacists.

**Logistic/Financial Aspects.** Despite promising developments in radiopharmaceutical chemistry, there are new challenges. The use of radioactive probes in, for example, clinical trials must strictly follow the many regulations in drug development and application. The synthesis of PET tracers for human application is subject to the same laws, and the need to follow GMP regulations brings serious challenges to the radiopharmacy.

Finally, the synthesis of PET tracers is by no means cheap. Costs include the radionuclide production in cyclotrons such as  $^{11}\text{C}$  and  $^{18}\text{F}$  and the subsequent preparative and analytical chemistry. Pharmaceutical companies have balked at the enormous investment for establishing cyclotron and radiochemistry laboratories, typically preferring to undertake contract research at academic centers that already possess the necessary technical expertise and infrastructure.

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### Notes

The authors declare no competing financial interest.

### Biographies

**Markus Piel** was born in Wiesbaden, Germany, in 1968. He studied chemistry at the Johannes Gutenberg-University (JGU) in Mainz and graduated in 1997. He received his Ph.D. in Nuclear Chemistry in 2001 at the JGU on the synthesis and evaluation of  $^{18}\text{F}$ -fluorinated NMDA antagonists. Currently, he is Senior Research Associate at the Institute of Nuclear Chemistry at the JGU, working on a wide variety of projects using different radioisotopes for labeling, with a focus on  $^{18}\text{F}$  and  $^*1$ . Since 2004, he is also Qualified Person and Head of the Quality control at the Institute of Nuclear Chemistry. His scientific research interests include the synthesis and evaluation of tracers for the CNS,  $\mu\text{PET}$  imaging in rodent models, and the development of new radiolabeling strategies.

**Ingo Vernaleken** was born in Bad Pyrmont, Germany (1970). He studied human medicine at the University of Saarland (graduated in 1998). He obtained his medical degree in 2000 at the Institute of Pharmacology and Toxicology in Homburg-Saar. His clinical engagement began in the Department of Psychiatry at the University of Mainz. Early in his medical career, he became involved in translational PET studies. In 2005, he became a senior physician to the Department

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Frank Rösch was born in Chemnitz, Germany, in 1955. He studied nuclear and radiochemistry at the Technical University Dresden, graduated in 1981, and obtained his Ph.D. in 1984. Subsequently, he had fellowships at the Laboratory for Nuclear Problems, Dubna, Sowjet Union, at the ZfK Rossendorf, Dresden, Germany, and, since 1992, at the Research Centre Jülich, Germany. In 1996, he was appointed Professor of Nuclear Chemistry at the Institute of Nuclear Chemistry at the Johannes Gutenberg-University Mainz, Germany. He is carrying out research in fundamental and applied radiochemistry and radiopharmaceutical chemistry. Apart from molecular imaging of the human brain, he also has an interest in radiometals and radionuclide generators and their application in the synthesis and clinical use of new radiotracers in the context of theranostics.

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## ABBREVIATIONS USED

2-<sup>18</sup>F]FDG, 2-deoxy-2-<sup>18</sup>F]fluoro-D-glucose; AADC, aromatic amino acid decarboxylase; AP, antipsychotic; BBB, blood–brain barrier;  $BP_{ND}$ , nondisplaceable binding potential; CGRP-R, calcitonin gene-related peptide receptor; CNS, central nervous system; CT, computed tomography; EPMS, extrapyramidal motor side effects; [<sup>18</sup>F]FDOPA, 6-<sup>18</sup>F]fluoro-L-dopa; FDA, food and drug administration; GABA,  $\gamma$ -aminobutyric acid; GLUT, glucose transporter; GlyT1, glycine transporter type 1; GMP, good manufacturing practice; HPLC, high-performance liquid chromatography; P-gp, P-glycoprotein; MAO-A, monoamine oxidase A; MRI, magnetic resonance imaging; NK1, neurokinin 1-receptor; NMDA, N-methyl-D-aspartate; PET, positron emission tomography; R&D, research and development; SGA, second-generation antipsychotics; SPECT, single-photon emission computed tomography; VTA, ventral tegmental area

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