Chapter 12

In Vivo Imaging of Dopamine Metabolism and Dopamine Transporter Function in the Human Brain

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Abstract

Positron emission tomography (PET) and single-photon emission computed tomography (SPECT) imaging of the dopamine system allow quantifying specific targets in the living animal and human brain. These methods are thus of great importance for translational brain research and have made it possible to identify and measure neurochemical changes associated with psychiatric disorders for the first time in history. The following chapter focuses on PET and SPECT imaging of psychotic disorders and addresses methods suited for imaging changes in extracellular dopamine levels and their relationship to dopamine metabolism and dopamine transporter function. Specifically, the chapter describes imaging with radiolabeled dopamine precursors (such as [^{18}F]DOPA) and the so-called "competition paradigms," where a change in extracellular dopamine elicits changes in radioligand binding to dopamine $D_{2/3}$ receptors. In addition to theoretical background, this chapter provides information on strengths and weaknesses as well as on practical aspects of these methods.

Key words Dopamine, DOPA, Psychosis, Schizophrenia, Amphetamine, PET, SPECT, [^{11}C]-(+)-PHNO

1 Introduction

The dopamine transporter (DAT) is an essential element in the regulation of intensity, duration, and spatial spread of brain dopamine signalling. DAT is the target of therapeutic and abused drugs such as methylphenidate, amphetamines, and cocaine. Imaging reductions in radioligand binding to DATs in the living brain using single-photon emission computed tomography (SPECT) or positron emission tomography (PET) has become an essential tool for aiding clinicians in diagnosing Parkinson's disease and other neurodegenerative disorders involving brain dopamine functions. Moreover, changes in DAT binding have been observed in populations with psychiatric disorders such as addiction or attention-deficit hyperactivity disorder (ADHD). The methods used for

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DAT imaging can be divided into those aiming at quantifying the amount of DAT protein accessible to radioligand binding, and those assessing DAT and dopamine function and metabolism. While quantifying DAT binding using PET or SPECT is comparably straightforward, measurement of DAT function in the living brain requires a more complex approach, and the interpretation of data relies on several assumptions that need to be tested and verified in animal experiments in vitro and in vivo.

Essentially, two different approaches have been used to study DAT function in the living human brain. One is using a radiolabeled version of the dopamine precursor dihydroxy-phenylalanine (DOPA), most commonly [^{18}F]DOPA. The other approach is to study the interaction of endogenous extracellular dopamine with postsynaptic receptors, most commonly dopamine D_2 and D_3 receptor subtypes. For this technique, a behavioural or pharmacologic intervention is used to alter extracellular dopamine levels. Typically, an increase in extracellular dopamine leads to reductions in radioligand binding, while a decrease in extracellular dopamine is associated with increased radioligand binding. This allows for an estimate of changes in extracellular dopamine levels in the living human brain. Although imprecise, the term "competition" or "displacement" study is frequently used to denote this method (see below for further discussion of this topic).

Both methods have provided significant insight into the pathogenesis of psychiatric disorders, first and foremost into altered dopamine transmission in psychosis or schizophrenia. In these conditions, macroscopic alterations of the brain—if present at all—are subtle or visible only after many years of active illness. Thus, with the notable exception of helping in the differential diagnosis to the so-called organic brain disorders, structural imaging methods have only limited diagnostic or prognostic relevance for clinical practice in the field of psychotic disorders. And as of yet, structural imaging has contributed little to our pathogenic understanding of these disorders.

In contrast, PET imaging of the dopamine system has clearly shown that dopamine function is altered in psychosis or schizophrenia. Dopamine synthesis and storage, as well as amphetamine-induced release of dopamine into the extracellular space are increased in psychotic patients. Moreover, studies in the prodromal phase of the illness have shown that alterations in brain dopamine function are detectable even before subjects develop clinical symptoms of psychosis [1, 2]. Thus, although costly and so far available to few specialized centers only, imaging methods based on the aforementioned techniques have the potential to become a useful clinical tool in the foreseeable future. Imaging could support clinicians in diagnostic and prognostic assessments or help stratifying patients for choosing specific treatment modalities.

Here, we try to provide a concise overview on basic principles of PET imaging and on possibilities and limits of studying dopamine metabolism and DAT function in the living human brain. This chapter is written for all those who are interested in the results of, but are not directly involved into PET studies on the dopamine system in the living human brain, or who think they may profit in one or the other way from some methodological background information.

2 Materials and Methods

2.1 Imaging Procedures

Radioligands are produced in a complex and resource-intensive process using a cyclotron for generating positron-emitting isotopes. For PET ligands used in studies of DAT function, the isotope is, at the time being, either flourine-18 ([^{18}F]) or carbon-11 ([^{11}C]). A practical advantage of [^{18}F]-labeled radiotracers over [^{11}C]-labeled ones is the slower decay (half-life of [^{18}F]: 109.8 min; [^{11}C]: 20.3 min), allowing for storage and transport for a limited time period. [^{11}C]-labeled ligands need to be produced on-site in close proximity to the PET scanning system. Target specificity and affinities to dopamine and non-dopamine receptors, brain penetration, protein binding, lipophilicity, metabolic stability and many other parameters need to be characterized extensively in vitro and in animal models before a radioligand is newly introduced to human PET imaging (see for example [3]). For a PET scan, the radioligand is injected as a bolus into a peripheral vein, usually over a time period of approximately 1 min. However, alternative methods exist, for example bolus plus constant infusion paradigms discussed later in this chapter. Depending on the radioligand and the imaging protocol, subjects need to lie supine in the scanner for 60–90 min, and sometimes for up to 3 h. The effective radiation dose injected is determined by the radioligand and its specific activity and usually ranges from less than 1 to up to 5 mSv (the average radiation dose from natural environmental sources to humans worldwide is approximately 2.4 mSv per year). Exposure to radioactivity is the limiting factor for the number of PET scans that can be performed in a subject for the purpose of research. A coincidence-detector within the PET camera is then used to detect and localize positron-annihilation events in the brain. The output of the so-called data reconstruction provides a temporo-spatial count-matrix into a four-dimensional image as output. During this process, data are corrected for heterogeneities in signal-attenuation brought about by anatomical variations in tissue composition. This correction is performed using an attenuation-matrix obtained in a so-called transmission scan performed directly before or after each PET scan. Furthermore, correction for radioactive decay is applied. Current high-resolution PET imaging systems can ascribe

decay-events to cubic volume units (voxels) with a side-length of 1.25 mm. However, this resolution is an ideal value reached at the center of the field-of-view only, and the reliability of the information depends on a variety of factors, such as image-reconstruction algorithms, partial volume effects, movement artefacts and so on. For improving anatomical accuracy of measurements, a magnetic resonance image (MRI) is acquired in a separate scanner and then "merged" with the PET image at a later point in time (see below). Combined PET/MRI imaging systems allowing for simultaneous MRI and PET measurements are a recent technological innovation available to few specialized research facilities only.

2.2 Image Analysis

PET data are analyzed using a broad variety of methods. Here, we confine the description to methods most commonly used for analysis of [^{18}F]DOPA and $D_{2/3}$ "competition" data. The fundamental principle of neuroreceptor (understood as general term including also DAT) PET is to use pharmacokinetic information acquired during a PET scan for deriving a set of parameters describing ligand–receptor interaction in the brain.

2.3 Preprocessing of Imaging Data

Procedures of PET image analysis may vary greatly according to PET and MRI systems used, anatomical targets structures, radioligands, and specific study aims. Here we describe procedures that are frequently used for DAT or dopamine $D_{2/3}$ receptor imaging. Usually, PET and MRI images are co-registered using linear iterative interpolation algorithms implemented into freely or commercially available software packages (e.g., Statistical Parametric Mapping; SPM). In study protocols using repeated scans, this step also allows to bring all image-sets of one subject into the same space. Usually, this intermodal brain image registration is attained using the so-called "rigid-body co-registration" algorithms, in which images, in order to yield the best possible overlay, are moved in space but are not deformed.

Another approach is used for direct voxel-wise comparisons of PET data from different subjects. First PET and MRI images are rigid-body co-registered and then transformed ("normalized") into a standard space, for example "Montreal Neurologic Institute (MNI) space." A standard space is an anatomical template created by averaging many individual images, usually MRI images. In standard space, anatomical structures have fixed coordinates allowing for comparison of results independent of study equipment and individual anatomical variations. The rationale for using MRI images for these steps is that MRI images contain much more and more accurate anatomical information than PET images. The transformation matrix is then used to normalize the respective PET images. This approach is widely used for processing parametric PET data (parametric maps), when a tracer kinetic model is fit to time–activity curve data on a pixel-by-pixel basis. The result is a quantitative image of the parameters of a physiological or biochemical model.

2.4 Region-of-Interest Analysis

Tissue composition, receptor population and other parameters of relevance vary greatly within the brain. However, the analysis of PET data makes the assumption of uniform conditions within a given volume. Traditionally, these volumes are denoted "region of interest" (ROI). ROIs are selected according to anatomical and functional criteria and are usually brain regions where the respective radioligand shows sufficient and reversible (within the time of a PET scan) binding. In imaging DAT function, ROIs usually comprise DAT and $D_{2/3}$ receptor rich regions such as the striatum and brainstem regions. In studies using PET cameras with high resolution, it is possible to image subdivisions of the striatum such as the ventral striatum (VST), putamen (PUT), caudate nucleus (CAU), and globus pallidus (GP). Resolution of current PET systems does usually not allow for a reliable distinction between the substantia nigra (SN) and the ventral tegmental area (VTA). Thus, these areas are usually analyzed in a combined brainstem ROI (SN/VTA). Moreover, high-resolution PET systems have lately been able to detect specific binding in small regions with only intermediate levels of dopaminergic target molecules, such as hippocampus or amygdala. For the analysis, decay-corrected regional radioactivity is used to measure pharmacokinetic behaviour of the radioligand in a given ROI. Graphically, this gives the so-called "time–activity curves" (TACs). These curves are then analyzed using a variety of methods, most frequently by iterative fitting to predefined compartmental models.

2.5 Time–Activity Curves (TACs) and Analysis of Binding Parameters

The primary outcome measures in neuroreceptor PET imaging are TACs. TACs are obtained by plotting decay-corrected regional radioactivity against time (see Fig. 1 for exemplary TACs obtained with the dopamine D2/3 receptor agonist radioligand [^{11}C]-(+)4-propyl-9-hydroxynaphthoxazine; [^{11}C]-(+)-PHNO; Fig. 2). The pharmacokinetic behaviour of the radioligand in a given ROI is used to derive a set of binding parameters describing pharmacological properties of the ligand–receptor interaction such as maximal binding capacity (B_{max}), affinity (given by the dissociation constant K_d), or, in case of [^{18}F]DOPA PET, the influx constant K_i.

Parameters are estimated by iterative fitting of PET data to a mathematical model known to adequately reflect pharmacokinetics and binding behaviour of the respective radioligand in the brain. Depending on the ligand, models need to take into account the exchange of radioligand between various tissue compartments, formation of radioactive metabolites and their diffusion or active transport between compartments, plasma protein binding etc. A scheme representing a so-called three-compartment model is depicted in Fig. 3. This model reflects imaging protocols using a so-called arterial input function, where blood is repeatedly collected from a peripheral artery (in most cases an artery at the wrist).

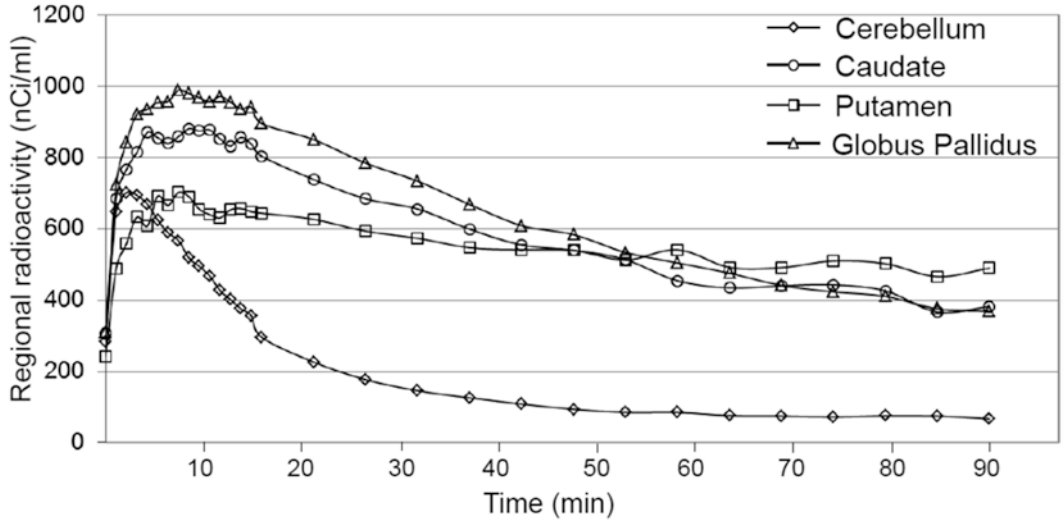

Fig. 1 Time–activity curves (TACs) for the dopamine $D_{2/3}$ receptor agonist-radioligand [^{11}C]-(+)-PHNO in striatal regions of interest (ROIs) and the cerebellum (reference region) in a healthy human subject

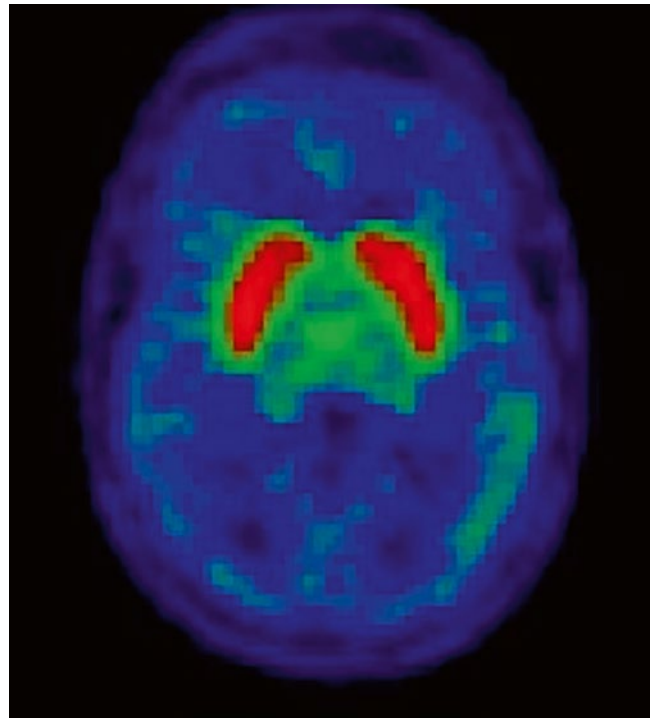

Fig. 2 Average image of a dynamic PET scan showing binding of the radioligand [^{11}C]-(+)-PHNO to dopamine $D_{2/3}$ receptors in the human striatum

Blood is analyzed for total and protein-bound radioligand concentration for obtaining free plasma radioligand concentration. Free plasma concentration is assumed being the true concentration delivered through the blood–brain barrier.

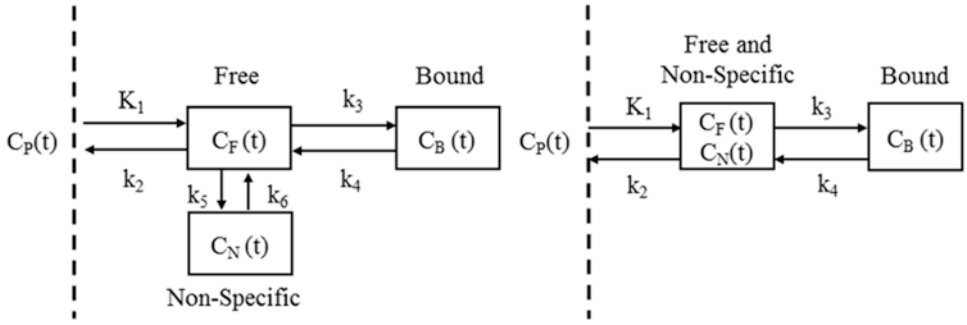

Fig. 3 Schematic of a three- (*left*) and two-compartment (*right*) model describing movement of a radioligand between compartments. The free radioligand fraction can be distinguished from nonspecific binding by using an arterial input function. This is not possible for a noninvasive reference region approach. These fractions are thus treated as one compartment assuming equal conditions in receptor-rich and reference regions. K_1 denotes the rate constant for transfer from plasma to tissue (unit: mL cm^{-3} min^{-1}). k_2, k_3, k_4, k_5, and k_6 denote rate constants for transfer into or out of the compartments as shown in the diagram (unit: min^{-1}). k_3 and k_4 reflect the ligand–receptor association (k_{on}) and dissociation (k_{off}) rate constants at a molecular level. And their ratio (k_4/k_3) is proportional to the in vitro dissociation constant K_d

2.6 Analysis Using Noninvasive Reference-Region Approaches

Since collection and analysis of arterial blood is somewhat painful for the imaged subject and cumbersome for the research team, noninvasive reference tissue models [4] are applied whenever possible. The PET signal in the brain reflects a mixture of free (unbound), nonspecifically bound, and specifically bound radioligand. A reference region is a brain region that contains no or negligible amounts of the targeted receptors (here DAT or $D_{2/3}$ receptors) but has a similar tissue composition and thus, comparable nonspecific binding. Free and nonspecific binding is collapsed into one compartment, and specific binding in the ROI is calculated using rate constants for free and nonspecific binding measured in the reference region. Feasibility and specifics of a reference tissue approach are determined for every radioligand in what is commonly called "modeling" of a radioligand. For the purpose of modeling, data obtained with an arterial input function (see for example [5, 6]) are used as a gold standard.

For dopamine imaging, the most commonly used reference regions are the cerebellar or occipital cortex, as they contain negligible dopaminergic innervation. The divergent pharmacokinetics of the $D_{2/3}$ receptor radioligand [^{11}C]-(+)-PHNO in $D_{2/3}$ receptor-rich striatal regions and the cerebellum can be easily recognized in Fig. 1. With respect to the cerebellum, it is an important detail to use cortical cerebellum with a certain distance to midline structures such as the vermis, as these midline structures have been shown to exhibit some degree of specific binding.

2.7 Parametric Analysis

The so-called "parametric" analysis methods treat every voxel in a PET image as ROI, i.e., kinetics of radioligand binding are

separately analyzed in every voxel in the brain [7]. After data preprocessing (anatomical normalization etc., see above), the behaviour of the radioligand can be compared between subjects and/or conditions at a single-voxel level. This approach has the advantage that it does not require a-priori an anatomical hypothesis that might, at times, conceal true functional connections. However, it conveys the disadvantage that information in single voxels is inherently noisy and prone to bias introduced by artefacts such as partial volume effects and others. Moreover, statistical methods become more complicated, not least because the number of statistical tests performed in a single analysis equals the number of voxels in the image. The methods for addressing the multiple-comparisons problem and other peculiarities are similar to those applied in functional magnetic resonance imaging (fMRI), and usually, parametric PET maps are analyzed using adapted software originally designed for fMRI. A frequently adopted approach is to combine parametric and traditional ROI analysis methods, e.g., by "masking" parametric maps in order to restrict the voxel-wise analysis to relevant brain areas.

2.7.1 [^{18}F]DOPA PET

After exogenous administration, DOPA is reversibly transported through the blood–brain barrier (BBB) by a transporter that DOPA is sharing with other amino acids [8]. This leads to equilibrium conditions between brain and plasma DOPA concentrations. Dopamine is formed from DOPA by decarboxylation via the enzyme aromatic amino-acid decarboxylase (AAADC, also known as DOPA-decarboxylase). The rate-limiting step in catecholamine synthesis under physiological conditions is hydroxylation of tyrosine via tyrosine hydroxylase. This step is no longer needed if DOPA is administered exogenously. Studies on [^{18}F]DOPA uptake and utilization thus depend on metabolic and transport events distal to tyrosine and tyrosine hydroxylation. Although AAADC is not specific for DOPA, its activity is highest in dopamine rich brain regions. Since dopamine no longer crosses the BBB, and since from a kinetic point of view, it is "trapped" in the brain compartment, AAADC plays a crucial role in the formation of the [^{18}F]DOPA signal in the brain. In neurons, dopamine transport between the extracellular and intracellular compartment depends primarily on DAT. Cytoplasmatic dopamine is transported into synaptic vesicles by the vesicular monoamine-transporter (VMAT), where it is stored and protected from degradation by monoamine oxidase.

Ultimately, the [^{18}F]DOPA signal reflects complex metabolic pathways in which DOPA is a precursor for epinephrine, norepinephrine, the so-called trace amines, or inert catabolic products. Consequently, DAT function is only one of several processes contributing to the [^{18}F]DOPA PET signal in the brain. DOPA (and [^{18}F]DOPA) is reversibly transported into an out of the brain (Fig. 4). A major metabolite of DOPA is *o*-methyl-dopamine (OMD). OMD is formed by methylation via catechol-*o*-methyltransferase (COMT), an

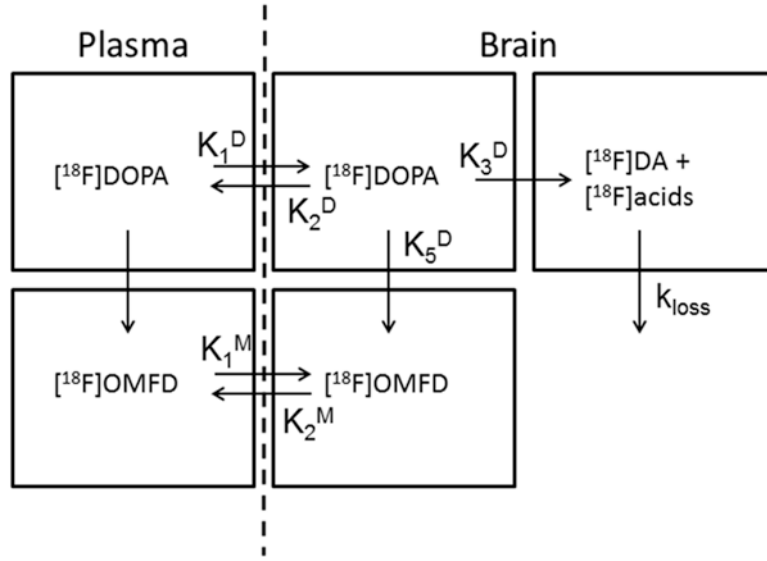

Fig. 4 Schematic of the compartmental model of [^{18}F]dihydroxy-phenylalanine ([^{18}F]DOPA) pharmacokinetics in the brain. [^{18}F]DOPA is transported from blood plasma into (K_1^D) and out of the brain (K_2^D). In blood and brain, [^{18}F]DOPA is metabolized by catechol-*o*-methyltransferase (COMT) to [^{18}F]-o-methyl-fluorodopamine ([^{18}F]OMFD) at the rate constant K_5^D. [^{18}F]OMFD reversibly crosses the blood–brain barrier at the rates K_1^M and K_2^M. In dopaminergic neurons, [^{18}F]DOPA is metabolized to [^{18}F]dopamine by the enzyme aromatic amino-acid decarboxylase (AAADC) at the rate constant K_3^D, indicating the activity of AAADC relative to the concentration of its substrate. [^{18}F]dopamine is stored in synaptic vesicles or metabolized to acidic degradation products leaving the brain by diffusion (k_{loss})

enzyme that inactivates dopamine in cortical brain regions where DAT expression is low. In the periphery, DOPA is metabolized by COMT in liver and blood cells. Thus, [^{18}F]DOPA PET studies are usually carried out after administration of a COMT inhibitor such as carbidopa. Carbidopa does not enter the brain and prevents signal loss by peripheral degradation of [^{18}F]DOPA. However, the presence of the [^{18}F]-coupled OMD isotope ([^{18}F]OMFD) in the brain, as well as the presence of radioactive dopamine degradation products should not be forgotten when interpreting [^{18}F]DOPA PET results.

Using an arterial input function and correcting for [^{18}F]DOPA metabolism, Cumming and colleagues [9] have shown that increased [^{18}F]DOPA uptake can be present together with increased [^{18}F]DOPA catabolism in patients with schizophrenia, indicating that the increase [^{18}F]DOPA uptake- and storage-capacity found in patients with schizophrenia in several independent studies may co-occur with increased dopamine degradation. Using the same analysis method, it has been shown that [^{18}F]DOPA turnover increases significantly after a single dose of methylphenidate [10].

2.8 Reference-Region Based and Linearized [^{18}F]DOPA Analysis Methods

As seen above, the brain signal measured with [^{18}F]DOPA PET is influenced by a number of factors and composed to varying degree by metabolites of DOPA and dopamine. Although the interpretation of the signal is not straightforward, there is a large number of studies carried out in various clinical populations using [^{18}F]DOPA and PET. Arterial sampling is not convenient and at times, painful for the research subject. Thus, most [^{18}F]DOPA PET studies use a reference region approach. Cerebellum and occipital cortex are nearly devoid of dopamine neurons and are thus frequently used as reference region. As first described by Hartvig et al. 1993 [11], [^{18}F]DOPA uptake can be conveniently analyzed using linearized methods similar to a Scatchard plot or, for PET analysis, a Gjedde–Patlak plot [12–14]. In this form of analysis, the slope of the linear regression corresponds to [^{18}F]DOPA uptake (or AAADC activity) in dopamine rich regions relative to the input into the reference region. Since the assumption that [^{18}F]dopamine is irreversibly "trapped" in AAADC-rich brain regions is progressively violated by dopamine metabolism over time, this form of analysis is limited to a relatively brief period after tracer injection (usually about on hour). However, reference region based methods have successfully been used in many studies on Parkinson's disease or schizophrenia, and they have given reliable and replicated results reflecting reduced (Parkinson's disease) or enhanced (schizophrenia) dopaminergic neurotransmission in clinical populations.

In summary, the signal measured using [^{18}F]DOPA and PET is a complex composite measure of several enzymatic and transport processes involved in DOPA transport and metabolism, and dopamine synthesis, uptake, vesicular storage, catabolism, and subsequent elimination from the brain. In part, the relative contributions of these processes can be quantified when using arterial plasma sampling during image acquisition. Nevertheless, also approaches using more practicable reference region-based analysis methods have provided important insight in alterations of the dopamine system in clinical populations.

3 Competition Studies

Symptom provocation studies using DAT blockers or releasers are classical paradigms in the research on the biological basis of psychotic disorders (see [15] for review). Long before the mechanism of action of these drugs was understood, it was known that high doses of amphetamines are able to provoke psychotic symptoms in healthy subjects, and that patients with psychotic disorders show behavioural super-sensitivity towards amphetamines or methylphenidate (in this case, behavioral supersensitivity in patients indicates de novo occurrence or worsening of preexisting psychotic symptoms at doses of methylphenidate or amphetamine that are inert or

induce only mild elevations in mood and energy when administered to healthy subjects). The insight that raising extracellular dopamine levels is an effect common to both drugs has significantly helped to shape the concept of psychosis as a hyper-dopaminergic state. In this context, it is important to bear in mind that enhanced dopamine release to DAT blockers or releasers is neither specific (see for example [16]) nor a necessary prerequisite for the presence of psychotic symptoms [17, 18]. Thus, the purpose of this and similar research methods is to reshape our understanding of the psychosis-syndrome and to aid stratification of patients for research and clinical purpose according to the underlying pathogenesis.

In contrast to some remarkable but not replicated early findings (see for example [19]), there is now wide agreement on the fact that there are no relevant changes in baseline availability of dopamine D_1 or $D_{2/3}$ receptors in patients with schizophrenia (due to a lack of suitable radioligands to low levels of expression, attempts to image dopamine D_4 and D_5 receptors have not been successful so far). Soon after the introduction of the benzamide $D_{2/3}$ receptor radioligand [^{11}C]raclopride into human PET imaging [20, 21], it was noted that raclopride binding was sensitive towards changes in the concentration of endogenous dopamine in the rodent brain [22, 23]. Initially, this was seen as a possible weakness for reliably quantifying $D_{2/3}$ receptors in the brain. However, the potential of this effect for imaging changes in dopamine levels in the living brain was soon understood [24, 25]. Since then, many studies have shown changes in receptor binding after pharmacological or behavioral manipulation of brain extracellular dopamine levels. In human studies, the most frequently adopted strategies for manipulating extracellular dopamine levels are to pharmacologically induce an increase in extracellular dopamine by administering methylphenidate or d-amphetamine, or to induce a decrease by administering a dopamine-depleting agent such as alpha-methyl para tyrosine (AMPT). Together with evidence from [^{18}F]DOPA PET imaging, PET studies showing enhanced d-amphetamine-induced reductions in $D_{2/3}$ receptor radioligand binding have contributed substantially to the fact that it is now widely accepted that there is enhanced dopamine transmission at least in a large proportion of patients with schizophrenia [1].

3.1 Theoretical Background of "Competition" Studies

Simultaneous measurements of changes in $D_{2/3}$ receptor radioligand binding and extracellular dopamine levels after d-amphetamine have shown a linear relationship between both measures suggesting that increased dopamine is indeed what causes decreased $D_{2/3}$ radioligand binding [26, 27]. These studies suggest that 1% decrease in radioligand binding is indicative of an increase in extracellular dopamine of approximately 40% [28]. However, data in these studies showed large variability, and the exact mechanism leading to these reductions in PET or SPECT experiments is not entirely understood.

While "competition" is frequently used as a cursory explanation, a series of experiments have shown that noncompetitive mechanisms contribute significantly to reductions in $D_{2/3}$ receptor radioligand binding after d-amphetamine administration [29, 30]. Pure competition reduces the affinity ($1/K_D$) of a ligand to a receptor without affecting the maximal number of binding sites (B_{max}). This principle holds true in studies reducing concentration of endogenous dopamine using depleting agents such as reserpine or AMPT [31, 32]. However, some observations on d-amphetamine induced reductions in radioligand binding are difficult to reconcile with a pure competition model. One example is that reductions in $D_{2/3}$ receptor radioligand binding outlast d-amphetamine induced elevations in extracellular dopamine [33, 34].

For disentangling d-amphetamine induced changes in affinity and changes in the number of $D_{2/3}$ receptor binding sites, Ginovart et al. [30] used a Scatchard approach for [^{11}C]raclopride PET in cats. A Scatchard plot is a linearized graphical analysis method depicting the relationship between the concentrations of free and bound ligands in a system. For the in vivo PET approach, Ginovart et al. used [^{11}C]raclopride with high and low specific activity. This study showed changes in $D_{2/3}$ receptor B_{max} *and* K_D after d-amphetamine administration, suggesting that d-amphetamine induced reductions in radioligand binding involve at least two different mechanisms.

In summary, the pharmacology of $D_{2/3}$ receptor "competition" studies is only partially understood, and the method is not suited for measuring absolute levels in extracellular dopamine. However, it yields fairly reliable estimates on relative changes in brain extracellular dopamine after behavioural or pharmacologic interventions.

3.2 Imaging Procedures and Data Analysis in "Competition" Studies

Measuring relative changes in extracellular dopamine implicates the need for a baseline value as comparator for the effects of the intervention. Basically, there are two different approaches used in the literature: One uses two PET scans, one without intervention (baseline) and another one with intervention—for example after d-amphetamine administration. The other approach makes use of a bolus plus constant infusion paradigm, where changes can be imaged in one scanning session.

In a two-scan approach, d-amphetamine is administered orally or intravenously before radioligand injection. These studies are thus pretreatment or blocking paradigms. Extracellular dopamine bound to $D_{2/3}$ receptors blocks radioligand binding and leads to reductions in specific $D_{2/3}$ binding in target ROIs. Attention needs to be paid if d-amphetamine leaves binding in the reference region (usually cerebellum) unaltered. This should be the case, as a prerequisite for a reference region is the absence of specific binding. Kinetic analysis assumes that delivery and washout rates in the

reference region are unaltered by d-amphetamine, or at least, that changes in target ROIs match those in the reference region. However, this needs to be ascertained for every radioligand used. In animal experiments, attention needs to be paid on possible interactions between d-amphetamine effects and the effects of anaesthesia. The outcome measure in reference region-based approaches usually is the so-called non-displaceable binding potential (BP_{ND}; [35]) defined as

$$BP_{ND} = B_{max} / K_D$$

where B_{max} indicates the maximal number of available binding sites, while K_D is an inverse measure for the affinity of the radioligand for the receptor. Under tracer conditions, BP_{ND} values are assumed to be linearly proportional to the number of binding sites. However, it is not possible to disentangle changes in affinity and receptor availability in a single PET scan (see above).

Relative changes in radioligand binding are usually reported as percent change in BP_{ND} values and calculated as

$$BP_{ND} = \left(BP_{ND\,d\ amphetamine} - BP_{ND\,baseline}\right) / \left(BP_{ND\,baseline}\right) \times 100$$

As mentioned above, d-amphetamine induced reductions in radioligand binding may last for several hours. It may thus be preferable to perform baseline scans before d-amphetamine scans. On the other hand, order effects, due for example to the effects of novelty and consecutive changes in dopaminergic tone in subjects undergoing PET scanning for their first time are better controlled for in a crossover study design with randomized scan order. In addition, possible carryover effects, i.e., $D_{2/3}$ receptor occupancy by the radioligand itself, should be considered in the study design. A [^{11}C]-(+)-PHNO PET in baboons [36] found significant residual occupancy in dopamine D_3 receptor-rich regions (ventral striatum, pallidum, substantia nigra / ventral tegmental area) at an interscan interval of 3 h. A recent study in humans [37] showed consistently lower [^{11}C]-(+)-PHNO binding in the second scan performed approximately 5 h after the first one. However, reductions were not statistically significant, and no relevant residual occupancy was found in other brain regions.

3.3 Bolus Plus Constant Infusion Paradigms

The outcome measures for quantification of radioligand binding to receptors are based on the assumption of equilibrium binding conditions, or else, a state where the net exchange between the plasma free fraction of the radioligand, nonspecific and specific binding are in steady state. Since this is not the case when the radioligand is injected as a bolus and its concentration changes at differing rates

in different tissues, mathematical compartmental modeling is used to infer concentration ratios and volumes of distribution. For some radioligands, however, it is possible to achieve "true" equilibrium conditions by first injecting the ligand as a bolus an then, in the course of the PET scan, supplying the ligand at a rate where concentration ratios between tissues remain constant. This method is usually denoted "bolus plus constant infusion" and can offer a series of advantages over conventional bolus PET imaging, for example simple and reliable quantification of binding parameters.

However, bolus plus constant infusion paradigm is not straightforward and sometimes technically challenging. The exact modalities for a bolus plus constant infusion need to be establishment experimentally for each radioligand, as depending on tracer kinetics, equilibrium binding is not always achieved during the time of a PET scan, especially when using short-lived isotopes such as [^{11}C]. If the concentration ratio is not truly constant but changes at a stable rate during a scan, this is denominated "pseudo-equilibrium" and can lead to errors in the estimates of concentration ratios. For radioligands washing out at differing rates in the different ROIs—as is the case for [^{11}C]-(+)-PHNO (see Fig. 1)—a given amount of radioligand constantly supplied will yield "true" equilibrium in some ROIs and pseudo-equilibrium in other ROIs. Still, even for [^{11}C]-(+)-PHNO, a radioligand known to bind to at least two relevant receptor populations (dopamine D_2 and D_3 receptors) with different kinetics, Lee et al. [38] recently succeeded in developing a bolus plus constant infusion paradigm with good reproducibility and reliable results.

For "competition" studies, the bolus plus constant infusion method has the big advantage that it becomes possible to measure dopamine release in on single scanning session. This reduces radioactivity exposure and time spent in the scanner for research participants and logistical and financial burden for researchers. Moreover, the method has the big advantage that it helps reducing the potentially biasing factors in a two-scan paradigm (various physiological and environmental changes occurring from one day to the other) to a minimum.

4 Notes

4.1 Studying Patients with Psychotic Disorders

Administering d-amphetamine to psychotic patients is sometimes considered to be ethically questionable, as it may temporarily intensify positive symptoms (delusions and hallucinations) and schizophrenic thought disorder. However, psychopathological ratings of symptom severity show an increase of a few percent only, and changes are self-limited in time and return to baseline after a few hours. Nevertheless, studies of this kind require a medical team with profound experience in treating psychotic patients. In our experience, a

low dose of d-amphetamine is usually well tolerated also by patients with psychotic disorders. And although positive symptoms and—according to our observations—in particular thought disorder intensify for a few hours, patients usually experience no particular distress under low-dose d-amphetamine. Rather, they report a general improvement in wellbeing. d-Amphetamine doses typically used in these studies are 0.3–0.5 mg/kg bodyweight. d-Amphetamine is administered either orally 1–2 h before scanning (in order to reach maximal d-amphetamine plasma concentrations during the PET scan) or intravenously immediately before, or in case of the so-called bolus plus constant infusion protocols (see above), during the scan.

Changes in positive symptoms are usually measured using the brief psychiatric rating scale (BPRS). The BPRS [39] is a brief scale rating overall psychiatric symptoms and contains specific items for psychotic symptoms. As ratings typically are performed several times within hours, the use of more comprehensive scales such as the positive and negative symptom scale (PANSS) is impractical.

A special challenge is recruitment and selection of patients whose symptoms are characteristic and intense enough for a clear-cut diagnosis (diagnosing schizophrenia can be difficult, especially during early stages of the disorder), but not as severe as to compromise patient safety or full understanding of study procedures and the ability to give informed consent. At therapeutic doses, antipsychotic medication induces significant occupancy of dopamine $D_{2/3}$ receptors (usually 60–80%; see for example [40]), making it impossible to disentangle occupancy induced by changes in endogenous dopamine and antipsychotic-induced occupancy. Thus, patients need to be either drug-naïve or drug-free for a sufficient period of time (due to slow elimination of certain antipsychotics from $D_{2/3}$ receptor-rich brain regions at least 2 weeks or longer [41]). Moreover, patients should be able to safely tolerate a delay of a few days in antipsychotic treatment, usually imposed by study logistics. Since a significant proportion of patients come to clinical attention several months or more after the first onset of psychotic symptoms, a delay in specific treatment of a few days usually imposes no significant discomfort or clinical risk to patients. Sometimes, however, it is clinically indicated and necessary to administer benzodiazepines for alleviating anxiety or psychomotor agitation. In order to avoid introducing bias, the benzodiazepine dose should be kept at a minimum and stable for the study period.

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