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PET Neurochemical Imaging Modes

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PET has deep roots in neuroscience stemming from its first application in brain tumor and brain metabolism imaging. PET emerged over the past few decades and continues to play a prominent role in the study of neurochemistry in the living human brain. Over time, neurochemical imaging with PET has been expanded to address a host of research questions related to, among many others, protein density, drug occupancy, and endogenous neurochemical release. Each of these imaging modes has distinct design and analysis considerations that are critical for enabling quantitative measurements. The number of considerations required for a neurochemical PET study can make it unapproachable. This article aims to orient those interested in neurochemical PET imaging to three of the common imaging modes and to provide some perspective on needs that exist for expansion of neurochemical PET imaging.
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Introduction

Neurochemical imaging with PET comprises a diverse set of molecular targets, radiotracers addressing those targets, experimental designs, and analysis options. For those not deeply rooted in the field, the permutation of variables can be overwhelming. Others have reviewed many aspects of PET neuroimaging,[1-5] but we focus on distinguishing features that enable what we term imaging "modes." Imaging modes, as we have termed them, are imaging experiment designs intended to answer specific chemical neuroscience questions. Each mode has specific considerations and constraints that enable biochemical information to be extracted from the imaging data. We highlight three common modes with the full recognition that other neurochemical imaging modes exist. Our goal with this article is to try and distill neurochemical imaging with PET to core elements that those new to the field can use as general guidelines for interpreting previous research studies in the literature.

The choice of imaging modes we highlight in this article is based on applications within neuropsychiatric and neurodegenerative disorders as they relate to neurotransmitter and pharmacologic research. We discuss a few additional biological targets and scenarios that are frequent in recent literature. Openly neglected are important areas of PET neuroscience including, for example, glucose metabolism,[6,7] other enzyme activity measurements,[8] and cerebral blood flow and oxygen metabolism.[9] The three modes we highlight deal with (1) measurement of protein density and density changes, (2) determination of drug occupancy and radiotracer competition, and (3) measurement of endogenous neurotransmitter release (Fig. 1). Within each of these three areas, we provide examples of experiments that demonstrate the general concepts and constraints. Finally, we discuss some perspective on what we feel are a few of the many unmet needs within neurochemical imaging with PET.

Characterization of Protein and Density Changes

The measurement of protein expression in the human brain and changes that occur in association with brain dysfunction is a common mode of PET neurochemical imaging. Many protein classes can be targeted with PET radiotracers, but we focus on just a few example protein classes that are common in PET neurochemical imaging, providing insight into the types of studies that are conducted for measuring protein and

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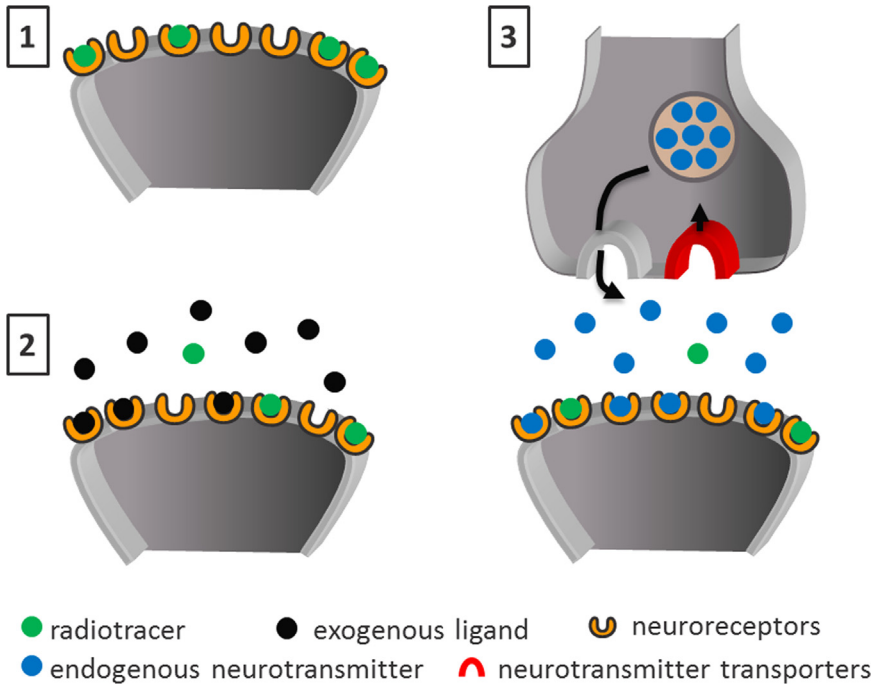

Figure 1 Three common modes for PET neuroimaging: (1) measurement of protein density and density changes, (2) determination of drug occupancy and radiotracer competition, and (3) measurement of endogenous neurotransmitter release.

density changes. Among the most common protein classes studied with PET are neuroreceptors, ligand transporters, and enzymes.

Neuroreceptors play a crucial role in neurotransmission and brain function regulation. Changes in the density of certain receptors or transporters have been associated with central nervous system (CNS) disease or aging process.[10] Receptor density can be measured ex vivo via postmortem autoradiography. However, in vivo quantitative imaging,[11] such as PET, of receptor density can help to elucidate disease mechanisms in the human brain.

For radiotracers to be useful in quantitative measurements of protein density, several criteria must be fulfilled to provide reliable results: (1) sufficient high dynamic range, which allows for accurate measurement of target density in both low- and high-distribution regions and to facilitate group comparisons (eg, patients with a disease vs healthy controls); (2) low methodological test-retest variability to ensure that differences observed between individuals can be attributed to the biological state of the brain; and (3) insensitivity to endogenous ligand binding, particularly in the "baseline or resting" state of the brain. Clearly, these considerations are not mutually exclusive and in general are highly radiotracer dependent. Nevertheless, we have outlined two primary experiments that serve as the basis for determining if a specific research question pertaining to protein density can be sufficiently answered with PET imaging.

First, an understanding of the intrasubject test-retest variability is important. By scanning a subject twice under the assumption that the biological state of the brain (protein level and availability of sites for radiotracer binding) has not changed, the difference in measurements provides the absolute minimum change that one could expect to quantify. Of course, in actuality, the effect size of a biological change must be much greater than the test-retest variability, given that PET sample sizes are often limited by scan costs. Generally, intrasubject variability is on the order of 10%-15%,[12] and although methodological and analysis improvements are shrinking this number, relatively small changes in protein density are currently difficult to measure. PET imaging of density has thus been most successful in scenarios where protein density changes are large (eg, $>50\%$).[13]

The second critical consideration (less often predetermined for radiotracers) when measuring density changes with PET provides evidence for insensitivity to endogenous state changes (eg, endogenous ligand-binding changes). For most mass action–driven PET analysis methods, receptor density is commonly represented by binding potential (BP), which is proportional to the number of binding sites (B_{max}) and inversely proportional to the dissociation constant (K_d) of the radiotracer for binding sites ($BP = B_{max}/K_d$).[14,15] If the radiotracer is in competition for binding sites, then only the available binding sites (ie, unoccupied) are measured (B_{avail}), increasing the complexity of absolute protein density (B_{max}) measurements with PET (see strategies later). When a radiotracer is insensitive to endogenous ligand binding, the aforementioned complexities can be negated, and changes in BP among subjects can more accurately be inferred as a protein density change (Fig. 2). Tests for sensitivity to endogenous release rely on the notion that protein synthesis rates are slow relative to changes in neurotransmitter (or endogenous ligand) concentration. A common experimental design relies on imaging modes (highlighted in the following two sections) that measure changes in neurochemistry. If it has been determined that a

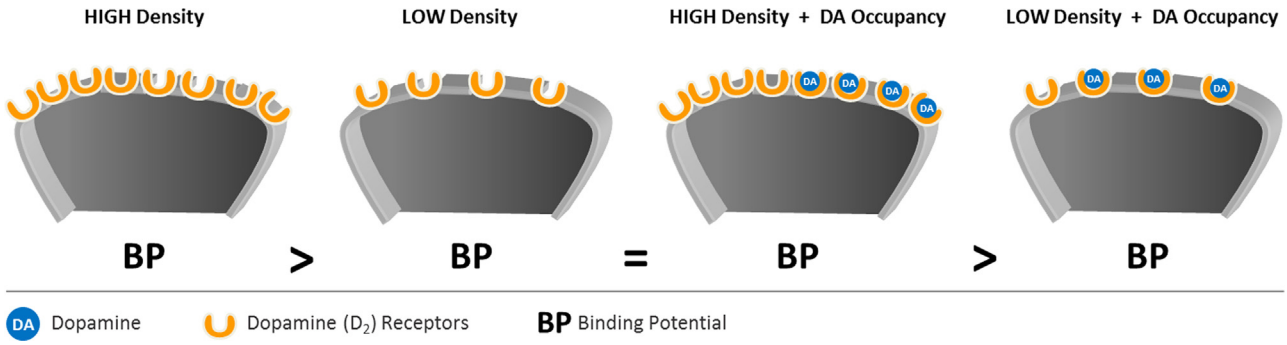

Figure 2 Effect of receptor density and endogenous ligand occupancy on the number of available binding sites for the radiotracer, binding potential (BP). Understanding the level of endogenous ligand binding is essential for determination of receptor density in vivo as both low density and high density with endogenous occupancy may result in similar BP levels.

particular radiotracer is insensitive to a competition mode, one can assume (albeit with some limitations) that PET signal differences are primarily protein density driven.

Several radiotracers for the serotonin (5-HT) system provide an example of insensitivity to endogenous ligand (serotonin release) and have therefore been used for determining protein density changes in healthy subjects vs subjects with a disease. We highlight two examples from this receptor class. [^{11}C]WAY-100635 and [^{11}C]DASB. [^{11}C]WAY-100635 is a high-affinity antagonist used for in vivo quantification of 5-HT$_{1A}$ receptor density.[16] It was observed that there was no decrease in binding of [^{11}C]WAY-100635 to 5-HT$_{1A}$ receptors after treatment with 5-HT–releasing agents (p-chloroamphetamine, fenfluramine, and methylenedioxymethamphetamine) or after depletion of 5-HT by treatment with 5-HT synthesis inhibitor (p-chlorophenylalanine) in rodents.[17] Therefore, a decrease in [^{11}C]WAY-100635 binding likely indicates a reduction in the density of 5-HT$_{1A}$ receptors. As another example, [^{11}C]DASB was developed for quantification of 5-HT transporter (5-HTT).[18-22] It is highly selective for 5-HTT with nanomolar affinity, has good dynamic range (V_T and BP), and low test-retest variability (<10% in all regions).[20,23] As with [^{11}C]WAY-100635, [^{11}C]DASB has shown insensitivity to drugs that stimulate serotonin release or depletion.[24,25] As seen from these two examples, the use of PET neuroimaging to measure protein density for dynamic systems (eg, neurotransmitter-receptor systems) leads to a convolved and potentially confounded interpretation. Protein density can certainly be a driving factor for PET imaging signal changes, but its overall contribution can be difficult to assess.

The association of PET signal changes with protein density changes is clearer in other cases where endogenous ligands are not in competition with the radiotracer. A primary example is amyloid aggregate imaging, which has been reviewed extensively.[26-28] Another example is the measurement of translocator protein (TSPO) expression with PET, which has been used as a marker of neuroinflammation for several diseases including Alzheimer disease,[29] Parkinson disease,[30] Huntington disease,[31] and ALS.[32] [^{11}C]PBR28 binds to TSPO, thereby quantifying microglia activation and subsequent neuroinflammation in healthy subjects and subjects with a disease.[33] The best evidence of PET signal association with TSPO (protein) density comes from supporting ex vivo analysis studies, for example, with immunohistochemistry.[34]

Clearly, the ability to measure protein density and changes in the living human brain is important, and we have only superficially addressed the radiotracers available for this imaging mode. In general, our advice is to avoid specifically attributing PET signal and signal changes to protein density without additional PET or ex vivo data to support a protein density–based interpretation.

Drug Occupancy and Radiotracer Competition

One of the more powerful modes of neural PET imaging has focused on determining occupancy of various psychoactive drugs. Within this context, PET has proven a useful tool for studying target engagement in vivo. These studies have made important advances in the field of neural drug development and neuropsychopharmacology, as they have provided evidence of brain uptake, specific binding to the target, and ligand-receptor dynamics.[35] Generally, the experimental design for determining drug occupancy or ligand displacement involves treatment with an exogenous ligand that directly competes with the radiotracer at the binding site. As drug occupancy with PET has been the focus of prior review,[36] it is not our intention to provide an exhaustive summary of this topic. Instead, we provide an update, and highlight the important parameters to be used as general guidelines for conducting drug occupancy or radiotracer competition studies with PET.

When several radiotracers are available for a particular target, selection is critically important to maximize sensitivity to competitive binding with the ligand. In addition to the standard criteria for CNS radiotracers,[5] we have highlighted radiotracer criteria for determining drug occupancy using a reversibly binding radiotracer:

1) High selectivity for the receptor or receptor subclass [eg, opioid receptor (OR) PET tracers that are extremely selective for μ vs κ or δORs].
2) High dynamic range and low nonspecific or nondisplaceable binding (ie, low off-target binding).

3) Occupancy of agonist drugs should be measured with an agonist radiotracer and antagonist drugs measured with an antagonist radiotracer to ensure competition for the same binding site.
4) Moderate to high in vitro affinity at the receptor (extremely high-affinity radiotracers with fast k_{on} and slow k_{off} may display poor sensitivity to ligand challenge).

In addition to radiotracer selection criteria, PET study design is equally important. Over the last several decades, there have been numerous advances in PET experimental design for determining drug occupancy and ligand displacement.[37] Conventional occupancy determination in vivo with PET can be conducted by pretreatment or coadministration with drug, paired with a bolus injection of the radiotracer. By analyzing radiotracer kinetics from several drug challenge experiments at various doses and comparing with baseline scans, we can determine the effective dose for occupying a certain quantity of receptors. A slightly different approach for measuring drug occupancy or ligand displacement has been conducted by administering a challenge after injection or infusion of the radiotracer. A common experiment for this method is a bolus/infusion (B/I) protocol, which requires administration of an initial bolus of the radiotracer followed by a constant infusion. The B/I method was designed to obtain equilibrium or steady-state concentration of the radiotracer in brain tissue. This method is less invasive than conventional bolus studies, because it typically does not require arterial blood sampling. The advantages of this method are (1) a single scan that can assess both baseline and challenge, (2) data analysis can be simplified in comparison with standard bolus experiments, and (3) venous sampling may be sufficient for determining free radiotracer in blood. In addition, B/I scan times may be shorter than bolus studies for kinetically slow radiotracers.[38] Achieving equilibrium with a B/I experiment is not always straightforward, and only some regions may achieve equilibrium. We describe in further detail the study designs and considerations of radiotracer properties for drug occupancy measurements.

Design Consideration for Drug Challenge Studies

Radiotracer administration can be varied to increase the sensitivity of PET imaging for a given drug competition measurement. We describe examples of both bolus and B/I study designs and some considerations for conducting each type of experiment. Although we focus on exogenous competition, there are conceptual commonalities that can be extended to endogenous ligand competition and are the topic of the next section.

Bolus Radiotracer Administration

For bolus competition studies, radiotracer criteria is less stringent than with B/I but must still meet the minimum CNS radiotracer requirements and follow the general guidelines we have outlined previously. In most instances, the first PET experiment consists of measuring normal or baseline levels of the drug's target with a bolus injection of the radiotracer for determining baseline kinetics and specific binding (BP_{ND}) or volume of distribution (V_T) levels.[39] In the follow-up experiment, the subject is treated with the drug, and a specific uptake time is allotted. Following uptake, which maximizes putative drug binding to the target, a bolus injection of the radiotracer is administered. Simply stated, a reduction in the outcome measure (eg, decrease in V_T or BP_{ND}) results in a change in available binding at the receptor, and the level of BP reduction is in direct correlation with drug occupancy at the administered dose. Of course, this type of experiment has many underlying assumptions, primarily among them, the radiotracer and drug exhibit mutually exclusive binding. Subtleties in the measure (changes in drug occupancy over the time course of imaging) are often ignored with the assumption that the drug binding and occupancy time course is slow relative to the imaging scan timeframe (eg, 60-90 minutes).

In general, the assumptions and occupancy estimates (as inferred by the kinetic differences between the two bolus scans) are reasonable and often correlated with less-extensive procedures for measuring drug levels, such as plasma drug concentration measurements. An example of using PET to validate plasma drug measurements to be used as an indicator of occupancy was demonstrated by Fowler et al.[40] In their report, drug occupancy levels from oral doses of the monoamine oxidase-A (MAO-A) inhibitor CX157 were determined with [^{11}C]clorgyline. To establish a dosing paradigm for clinical efficacy, they correlated drug occupancy measurements from PET with plasma drug levels, to validate plasma sampling as a biomarker for determine drug occupancy during clinical studies. It was determined that this method provided excellent correlation between PET occupancy measurements and plasma concentration.

Another example of validating methods or assays with PET drug occupancy studies involved determining the in vivo selectivity of an opioid antagonist drug—LY2795050. This drug is a part of a new class of kappa OR (KOR) antagonist drugs and was reported to have good selectivity for KOR over the mu OR (MOR) (36:1 KOR:MOR) in a cellular assay.[41] To determine the in vivo selectivity for this drug, a PET occupancy study was conducted in rhesus monkeys, using radiolabeled [^{11}C]LY2795050 (KOR radiotracer) and [^{11}C]carfentanil (MOR radiotracer) to assess opioid subtype selectivity (KOR:MOR).[42] Animals were treated with the KOR antagonist drug LY2795050 at six doses (1.6-400 μg/kg, intravenously) followed by a bolus injection of either [^{11}C]LY2795050 or [^{11}C]carfentanil. Animals underwent a 120-minute dynamic PET scan with arterial blood sampling. Following kinetic analysis of the data, it was estimated that the effective dose of LY2795050 achieved 50% MOR occupancy (ED_{50}^{MOR}) at 119 μg/kg and 50% KOR occupancy (ED_{50}^{KOR}) at 15.6 μg/kg. Based on this assessment, it was determined that the in vivo selectivity of this KOR antagonist was 7.6:1 (KOR:MOR), a substantial difference from the previous in vitro selectivity data (36:1

KOR:MOR), highlighting the power of this in vivo assessment with PET.

B/I Radiotracer Administration

Since the first drug challenge PET studies, new experiments have been used to determine binding changes from exogenous or endogenous stimuli. The most common example is radiotracer displacement studies that involve the administration of a challenge *after* the radiotracer has reached equilibrium with a B/I administration. With a B/I study design, intersubject variability can create challenges, as radiotracer kinetics and equilibrium may differ from subject to subject. Regardless, this method has demonstrated its sensitivity to pharmacologic challenge and typically requires only a single scan for obtaining baseline and postchallenge radiotracer kinetics. For determining infusion parameters, the radiotracer should be infused at a rate that obtains equilibrium in the brain region of interest. Analysis of the PET data can be accomplished by taking the ratio of the radioactivity between the target region and a reference region before and after challenge; thus, a percentage change in BP_{ND} can be quantified. Validation of the B/I method has been established for several PET radiotracers including, but not limited to, raclopride,[43] cyclofoxy,[44] carfentanil,[45] and altanserin.[46] A B/I paradigm has been successfully used to quantify direct drug occupancy targeting the dopamine system.[47,48]

Manipulation of radiotracer administration can have a marked effect on the ability to detect neurochemical changes in the brain and the bolus, and B/I methods are only the beginning of what will come. For example, a novel multi-infusion method was recently developed at Yale University, which allows for assessment of B_{max} and K_d in vivo.[49] Continued development in this area will lead to a greater ability to study neurochemistry with PET and will improve our ability to study both exogenous and endogenous stimuli.

Endogenous Neurotransmitter Release

Measuring changes in the level of endogenous neurotransmitters has enabled another important mode of PET imaging. This mode follows similar principles to exogenous occupancy studies, in that, a change in neurotransmitter levels causes a change in occupancy at the receptor, leading to a change in BP measured with PET.[50] This mode involves a release or change of an endogenous ligand induced either from a functional task[51-54] or by indirect pharmacologic challenge (eg, amphetamine for increase in DA release).[55] The most important principle for measuring endogenous neurotransmitter level changes with PET is radioligand sensitivity to competition with the endogenous ligand. To date, a limited number of receptor classes have been successful for this PET measurement (dopamine, and limited success with opioid and serotonin receptors), and only a few radiotracers have been capable of measuring changes in synaptic transmission in the living brain. The dopamine targeting radiotracers have been, by far, the most well-studied class, and [^{11}C]raclopride is the primary radioligand chosen for $D_{2/3}$ studies. Although these PET study designs differ slightly from drug occupancy measurements, they tend to follow the same experimental methods as outlined in previous sections. A bolus radiotracer administration with a two–PET scan paradigm (baseline and challenge) has been commonly used to determine receptor occupancy changes by endogenous ligand release, for example, endogenous opioid peptide release with experimental pain.[56] A B/I paradigm has also successfully quantified endogenous dopamine [57-59] and serotonin[60] release through indirect pharmacologic challenges. Studies in humans have also demonstrated the utility of a B/I method for detecting endogenous ligand release in response to tasks or stimuli.[61,52]

In addition to methodological overlaps between drug occupancy and endogenous ligand level measurements, the radiotracer selection criteria from previous modes can also be extended to measurements of endogenous neurotransmitter release. Building on those guidelines, a key requirement of an ideal radiotracer to detect endogenous neurotransmission is the pharmacokinetic parameters of the radiotracer. A radiotracer with a relatively fast dissociation rate would be more sensitive to synaptic neurotransmitter release owing to rapid adjustment of tracer-target binding from changes in neurotransmitter concentration. As an example of these characteristics, an early report measured binding changes of [^{11}C]raclopride in a bolus blocking study to detect changes in dopamine release following indirect pharmacologic challenge.[55] In this study, methylphenidate (0.5 mg/kg, intravenously) was administered to human subjects followed by a [^{11}C]raclopride bolus to assess the changes in BP between baseline and challenge scans. At a dose of 0.5 mg/kg, the subsequent stimulant-induced dopamine release resulted in a 23% decrease in [^{11}C]raclopride binding. Reiterating the necessity for high-sensitivity radiotracers for measuring endogenous ligand levels, it is important to note that a 23% decrease is only 13%-18% more than the test-retest variability (5%-10% for [^{11}C]raclopride). With human clinical research studies, small changes can be difficult to detect with small subject numbers, and it is costly if additional subject recruitment and scanning must be performed. Since this early report, measuring changes in endogenous dopamine with PET has been one of the most heavily investigated areas and the focus of several reviews.[63] Despite the advances in the detection of endogenous dopamine release, only a few other neuroreceptor systems have demonstrated success in measuring fluctuations in endogenous ligands, and with only limited success thus far.

An interesting direction within endogenous-release PET measurements is the ability to measure pulses of neurotransmitter release.[64,65] Currently, imaging-tracer methods only allow for detection of a single neurochemical release (Fig. 3B, only a single decrease in tracer to target binding) and fail to measure multiple, consecutive releases of neurotransmitters (Fig. 3C). Multiple releases of the neurotransmitter with high time resolution during a single PET scan is a powerful technique,[66,67] but it remains a challenge for most receptor systems and neurochemical release protocols. To help address this challenge, there is a need to design a new generation of radiotracers with faster protein association and

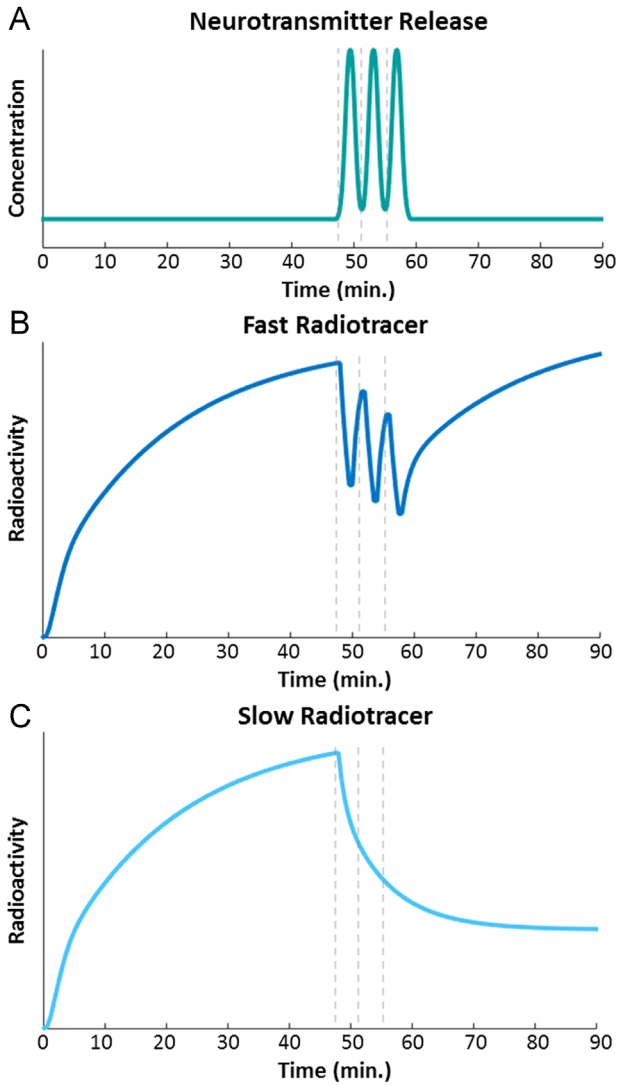

Figure 3 Computational simulation comparing the kinetics of slow- and fast-binding radiotracers and subsequent response to consecutive neurotransmitter release. Fast radiotracers (with large k_{on} and k_{off}) respond quickly to multiple endogenous neurotransmitter releases and depletions due to the rapid radiotracer "displacement" and "rebinding" to the target. Slow radiotracers can generally only respond to the first surge, if at all. (Color version of figure is available online.)

dissociation kinetics (and analysis methods to accompany their use). A radiotracer with a greater k_{off} (lower residence time at the target), can in theory, respond more quickly to endogenous neurochemical surge. Clearly, a balance needs to be struck between low residence time and affinity (often closely associated), as radiotracers with low affinity might lead to low signal-to-background ratio.

Summary

The development of PET studies for measuring neurochemical changes requires significant effort and extensive validation of protocols. We have outlined the three most common modes of brain PET as it applies to neuropsychiatric and neurodegenerative disorders related to neurotransmitter and pharmacologic research. This review serves as a general guideline for studying (1) protein density and change, (2) drug occupancy and radiotracer competition, and (3) endogenous neurotransmitter release, with PET. The examples provided illustrate the requirements and considerations when attempting to use each mode. Protein density measurements with in vivo imaging, such as PET, provide a powerful tool for monitoring disease progression, response to treatment, and diagnosis. Intersubject comparisons of protein density changes with PET rely on the principle that changes in radiotracer binding account for changes in protein density. Typically not emphasized are the assumptions made for defining a change, the methodological criteria used for conducting such studies, and the challenges that are encountered. We have outlined the criteria that are most often discussed and accounted for when designing protein density measurements with PET. In addition, drug occupancy studies comprise a major component in neurochemical PET studies, and this mode has seen considerable advancement over the years. Currently, two method designs predominate this field and have been used for determining drug occupancy with various neuroreceptors. These include a two-bolus design, measuring baseline kinetics in the first scan, and challenge kinetics in the second scan following drug administration. In addition, B/I paradigms have seen considerable success and offer unique advantages over the two-bolus method. The guidelines mentioned provide criteria for each mode and the importance of radiotracer selection on sensitivity to these measurements. Measuring changes in endogenous ligands with PET has been successful with the dopamine system but has limited success in other neuroreceptor classes mainly because of insufficient radiotracer properties. There has been interest in developing radiotracers with specific kinetic properties for detecting consecutive release of neurotransmitters, but this area is still in the investigational stages. As new advances in radiotracer design and PET instrumentation evolve, the multitude of different studies conducted within each mode will broaden, leading to a better understanding of neurobiological and neurochemical mechanisms.

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