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News and views on in vivo imaging of neurotransmission using PET and MRI

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Abstract

Molecular neuroimaging with PET is an integrated tool in psychiatry research and drug-development for as long as this modality has been available, in particular for studying neurotransmission and endogenous neurotransmitter release. Pharmacologic, behavioral and other types of challenges are currently applied to induce changes in neurochemical levels that can be inferred through their effects on changes in receptor binding and related outcome measures. Based on the availability of tracers that are sensitive for measuring neurotransmitter release these experiments have focused on the brain's dopamine system, while recent developments have extended those studies to other targets such as the serotonin or choline system. With the introduction of hybrid, truly simultaneous PET/MRI systems, *in vivo* imaging of the dynamics of neuroreceptor signal transmission in the brain using PET and functional MRI (fMRI) has become possible. fMRI has the ability to provide information about the effects of receptor function that are complementary to the PET measurement. Dynamic acquisition of both PET and fMRI signals enables not only an *in vivo* real time assessment of neurotransmitter or drug binding to receptors but also dynamic receptor adaptations and receptor-specific neurotransmission. While fMRI temporal resolution is comparatively fast in relation to PET, the timescale of observable biological processes are highly dependent on the kinetics of radiotracers and study design. Overall, the combination of the specificity of PET radiotracers to neuroreceptors, fMRI signal as a functional readout and integrated study design promises to expand our understanding of the location, propagation and connections of brain activity in health and disease.

Keywords

PET; fMRI; Receptor imaging; Neurotransmitter dynamics; Dopamine

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Conflicts of interest

The authors declare no conflict of interest.

Neurotransmitters are crucial in regulating brain activity and thus cognition and behavior. Signal transduction in the brain across neurons is accomplished by the release and binding of neurotransmitters, which leads to the activation or inhibition of specific neuronal pathways. Neurotransmitters thus play an important role in modulating the brain's activity at the molecular level. They are also important for maintaining a homeostatic balance of neuronal activity for healthy brain function. If levels of neurotransmitters are too low or too high, this results in brain dysfunction as observed in a range of neurodegenerative or psychiatric disease states. Hence, observing neurotransmission in the living brain is important for our understanding to brain function in health and disease.

Using positron emission tomography (PET), it is possible to image a range of neuroreceptor targets in the brain with high specificity. Imaging neuroreceptors with PET is one of the few methods that enable imaging of receptor binding *in vivo* in a non-invasive manner. Several different approaches can be taken when imaging changes in receptor binding using PET. In order to image neurotransmission, different types of challenges and interventions have been made to image changes in receptor binding that can infer neurotransmitter or drug binding. These include the use of drug challenges, behavioral modulation, disease states or direct stimulation. Each method has provided insight into changes in brain function that occur over time.

Brain imaging with functional magnetic resonance imaging (fMRI) has become a tool to explore how the brain processes information in the neurosciences and beyond. Together with advances in our understanding of brain anatomy, fMRI methods provide the ability to record changes in brain activity with a spatial resolution at the submillimeter level and a temporal resolution of seconds or less. While fMRI measures a hemodynamic response that is secondary to neuronal activation, it can be used to image the functional response of neuroreceptors to drugs or stimuli that target specific receptor sites, also termed pharmacological MRI (phMRI¹). fMRI can thus provide information about the effects of receptor binding that is complementary to the PET measurement and expands our understanding of the location, propagation and connections of brain activity.

In this review, we provide an overview of PET radiotracers that have demonstrated sensitivity to neurotransmitter release, and discuss the types of challenges and interventions that can be used to elicit neurotransmitter release. We will then discuss how fMRI has measured receptor-specific signals and how the simultaneous use of PET and fMRI can advance our understanding of receptor-specific neuronal transmission in the brain.

I. PET radiotracers that have enabled imaging of neurotransmission

PET radiotracers are now available for many targets in the brain, including various receptor types and presynaptically located transporters. To date, the main class of radiotracers shown to be sensitive for endogenous neurotransmitter release is specific to the dopamine receptor system (for an overview see for example Finnema et al. 2015²). Current research indicates that novel radiotracers developed for the serotonin (5-hydroxytryptamine, 5-HT), the opioid and the cholinergic system are also sensitive for changes in extracellular concentrations of their respective neurotransmitters.

Dopamine Receptors

Historically, brain dopamine has been the most extensively studied neurotransmitter and the prototype system for which changes in extracellular concentrations have been evaluated *in vivo* using PET. Several radiotracers for both the excitatory D1-like receptors (D1/D5) and the inhibitory D2-like receptors (D2/D3/D4) have been tested for sensitivity to endogenous dopamine in animals or humans using ^{11}C or ^{18}F -labeled compounds. The substituted benzamide derivative [^{11}C]raclopride has consistently been shown to display changes in binding which are consistent with dopamine competition³. More recently, antagonist radiotracers with higher D2 receptor affinity compared to raclopride, such as [^{11}C]FLB457 and [^{18}F]fallypride, have been used to investigate extrastriatal brain regions, where the density of D2 receptor is much lower than in the striatum. These studies showed reduced cortical radioligand binding, albeit to a lesser extent than striatal [^{11}C]raclopride displacement, that indicated that these radiotracers may allow for monitoring of changes in cortical synaptic dopamine concentrations when endogenous dopamine was available for release, also in clinical experiments⁴. The changes in radiotracer binding were proportional to changes in dopamine concentrations as measured in microdialysis experiments; findings that reflect modulation of dopamine release into the synaptic gap instead of inherently reflecting changes in the extracellular fluid dopamine concentration across drugs with different modes of action².

To further increase the sensitivity of radiotracer binding to endogenous dopamine, agonist radiotracers were developed for D2/D3 receptors including [^{11}C]PHNO, which preferentially binds to the high-affinity state of receptors⁵. This mechanism of action has been suggested to be more susceptible to the endogenous agonist than the binding of antagonist tracers, which do not discriminate between the low- and high-affinity states. Indeed, agonist radiotracers have been shown to be more sensitive to changes in extracellular dopamine concentrations than [^{11}C]raclopride, also in humans⁶. Furthermore, the use of [^{11}C]-(+)-PHNO offers the possibility of estimating *in vivo* endogenous dopamine levels at D3 receptors in extrastriatal regions⁷; however, the exact mechanism of the increased sensitivity of agonist radiotracers to dopamine is a matter of debate and warrants further evaluation.

In contrast to the D2/D3 receptor tracers, D1 receptor antagonist tracers such as [^{11}C]SCH23390 and [^{11}C]NNC-112 have not appeared to be sensitive to acute changes in extracellular dopamine concentrations^{8,9}. This might be due to the fact that dopamine has significantly lower affinity to the D1 receptor, which predominantly exist in the low-affinity state, than D2 receptor in the striatum^{10,11}. Important to note here is that the changes in the binding potential are typically interpreted according to a competition model, e.g., reflecting a change in dissociation rate constant K_D , but several observations suggest a contribution of dopamine receptor internalization as well².

Serotonin system

Similar to radiotracers that target the dopamine and noradrenaline transporters, radiolabeled-markers for the serotonin transporters, e.g. [^{11}C]DASB were used for the determination of transporter occupancy by reuptake inhibitors^{12,13,14,15}. Interventions to either elevate or

deplete extracellular serotonin, however, did not affect [^{11}C]DASB binding in humans¹⁶. Increases in synaptic serotonin concentration have also not or not consistently been shown to influence binding of the high-affinity 5-HT_{1A} receptor ligand [^{11}C]WAY100635, or other 5-HT_{1A} receptor antagonistic tracers such as [^{18}F]MPPF, [^{18}F]FPWAY or [^{11}C]CUMI-101, possibly due to the effects of 5-HT_{1A} autoreceptor activation². Antagonist radiotracers such as [^{18}F]setoperone, [^{18}F]altanserin, and [^{11}C]MDL 100907 for 5-HT_{2A} receptor have generally not shown sensitivity to endogenous serotonin release in PET studies. However, preclinical models have indicated that the novel 5-HT_{1B} receptor tracers [^{11}C]P943 and [^{11}C]AZ10419369 are sensitive for changes in endogenous serotonin concentration¹⁷, thus making 5-HT_{1B} receptor radiotracers interesting for the measurement of serotonin release¹⁸.

Other recent initiatives include work with the 5-HT₄ receptor antagonist [^{11}C]SB207145¹⁹ and the 5-HT_{2A} receptor agonist [^{11}C]Cimbi-36. Initial evaluation of [^{11}C]Cimbi-36 demonstrated serotonin sensitivity with changes that are detectable with PET when the serotonin release is sufficiently high²⁰. These results warrant further evaluation of [^{11}C]Cimbi-36 in humans to confirm the potential of agonist radiotracers for PET measurement of changes in extracellular serotonin concentrations.

Noradrenaline receptors

According to preclinical PET data, [^{11}C]yohimbine binding could be used as a surrogate marker of noradrenaline release *in vivo* in different brain regions expressing α_2 adrenoreceptors²¹. This has yet not been investigated in humans though recent results confirm the usefulness of the tracer [^{11}C]yohimbine for mapping and quantifying α_2 adrenoreceptors in the living human brain²². PET tracers for imaging central adrenoreceptors such as [^{11}C]yohimbine and [^{11}C]mirtazapine also do not distinguish between subtypes of α_2 , i.e. α_2A and α_2C adrenoreceptors. Recently, [^{11}C]ORM-13070 has been identified as a suitable radiotracer candidate for imaging α_2C adrenoreceptors and changes in extracellular noradrenaline concentrations in humans^{23,24}.

Acetylcholine receptors

Acetylcholine exerts its effects via metabotropic muscarinic receptor (mACh) and ionotropic nicotinic receptors (nACh). The M2 mACh receptor subtype selective agonist [^{18}F]FP-TZTP has shown sensitivity to acetylcholine in monkeys and humans²⁵. [^{11}C]AF150(S), which is an M1-selective mACh receptor agonist radiotracer also revealed preliminary hints for sensitivity to assess changes in extracellular acetylcholine concentrations²⁶ but this has not been further evaluated, or translated to humans so far. Other radiotracers that are subtype non-selective, such as ^{11}C -labelled (+)3-MPB, (+)3-EPB, (+)3-PPB, and NMPYB have been tested showing either no ((+)3-MPB, NMPYB) or significant changes ((+)3-EPB, (+)3-PPB) after increases in endogenous acetylcholine levels².

Besides the non-selective agonist [^{11}C]nicotine, which has relatively high nonspecific binding, PET tracers have been developed and investigated for imaging nACh receptor subtypes in the brain, in particular the most abundant $\alpha_4\beta_2$ and α_7 subtype. The $\alpha_4\beta_2$ agonists 2-[^{18}F]FA and 6-[^{18}F]FA have been available for PET studies in humans^{27,28}, however, 2-[^{18}F]FA has slow brain kinetics which hampers its use for clinical PET studies.

Novel radiotracers for imaging $\beta 2^*$ (*denotes the presence of other subunits in the receptor composition, i.e. $\alpha 4\beta 2$, $\alpha 6\alpha 4\beta 2$, and $\alpha 6\beta 2$) with faster kinetics and favorable binding parameters that have already been tested in humans include [^{18}F]AZAN²⁹, [^{18}F]nifene³⁰, (–)-[^{18}F]flubatine³¹ and (+)-[^{18}F]Flubatine³².

Glutamate and GABA transmission

Whereas the interaction between monoaminergic neurotransmitters and radiotracers is assumed to involve direct competition at the orthosteric binding site (potentially complicated by receptor trafficking), some radiotracers bind to an allosteric site on a receptor and act as positive or negative allosteric modulators of binding affinity (for review see for example Slifstein and Abi-Dargham, 2017)³³. Available PET tracers for imaging amino acids, i.e. L-Glutamate as an important excitatory neurotransmitter and GABA as the principle inhibitory compound in the CNS, bind to these allosteric sites.

With regard to ionotropic glutamate receptors, few radiotracers have been evaluated so far for PET imaging of the N-methyl D-aspartate (NMDA) receptor, to which ketamine binds to and exerts most of its actions. The methylguanidine derivative [^{11}C]GMOM has been used successfully to quantify NMDA receptor binding in both preclinical models and humans³⁴ but findings on glutamate release are pending. Other examples of radiotracers that have shown *in vitro* specificity for NMDA include [^{11}C]-CNS5161 and [^{18}F]GE-179. However, *in vivo* specificity for NMDA receptors still needs to be validated in these cases. More promising progress has been made towards PET imaging of metabotropic glutamate receptor subtypes, in particular the mGlu5 receptor subtype with [^{11}C]ABP688 as a radiotracer appearing sensitive to glutamate release³⁵. [^{18}F]FPEB and [^{18}F]SP203 have been demonstrated as mGlu5 receptor [^{18}F]-labelled PET radiotracers^{36,37}, but not yet for their ability to detect changes of endogenous glutamate levels.

Changes in synaptic GABA levels have been assessed in humans with radiotracers for ionotropic GABA_A and benzodiazepine binding sites, i.e. [^{11}C]flumazenil, and [^{11}C]Ro15-4513 as a PET ligand with high affinity for $\alpha 5$ -subunit-containing GABA_A receptors with detecting either small changes³⁸ or markedly reductions³⁹. These differences might also be explained by [^{11}C]flumazenil acting as a weak partial agonist and [^{11}C]Ro15-4513 being a partial inverse agonist at the benzodiazepine binding site².

Opioid system and sigma (σ) receptors

Opioid peptides act through four subtypes of G-protein-coupled opioid receptors (μ -, κ -, δ -, and nociception receptors), which have been studied using either [^{11}C]carfentanil for imaging μ -opioid receptors or the non-selective antagonist [^{11}C]diprenorphine (see II.). Although formerly thought to be a type of opioid receptor, pharmacological testing indicated that the σ -receptors were activated by drugs completely unrelated to the opioids, and their function was unrelated to the function of the opioid receptors. The $\sigma 1$ receptor is a chaperone protein located at the interface of the endoplasmic reticulum and the mitochondrion⁴⁰. It regulates several functional proteins including D1 and D2 receptors through oligomerization and is increasingly implicated in the pathophysiology of virtually all major central nervous system disorders^{41,42}. Recently developed, highly selective tracers

like (S)-(-)- and (R)-(+)-[¹⁸F]fluspidine⁴³ appear to be suitable for σ 1 receptor imaging in humans and are currently under clinical investigation as a promising drug treatment target.

Histamine H3 receptors

Finally, there is a growing interest in studying histamine H3 receptors as a drug target for several neuropsychiatric disorders. The histamine H3 receptors are presynaptic neuroreceptors that inhibit the release of histamine and other neurotransmitters. There are several H3 receptor tracers under development, e.g., [¹¹C]TASP0410457⁴⁴ and [¹¹C]MK-8278⁴⁵; of which [¹¹C]GSK189254 has recently shown the ability to image and quantify drug-occupancy in man⁴⁶.

II. Types of challenges that modulate neurotransmission and neuroreceptor binding

Pharmacological challenges

One of the most effective ways to modulate brain activity through receptor signaling in real time is the use of acute exogenous drug challenges and observe their effect on receptor availability and occupancy. There are several different routes through which drugs can be administered acutely and the type of drug challenge determines not only receptor binding but also secondary effects at the synapse and downstream signaling.

Direct target effects of exogenous drugs—Changes in binding at the receptor can be induced by binding of drug that is in direct competition with the radiotracer, such as direct agonists or antagonists. This method is also commonly used in blocking studies to evaluate specific binding of the radiotracer or a drug of interest. For example, the effect of direct binding of exogenous drugs has been used to evaluate antipsychotic drugs, i.e. D2 receptor antagonists, and their therapeutic window⁴⁷. Using relevant PET radiotracers, it has been established that clinically effective doses are often between 70–90% of D2 receptor occupancy⁴⁸. Figure 1 provides an example of [¹¹C]raclopride binding that results in dose-dependent binding of an injected D2/D3 agonist drug (quinpirole) in competition with the radiotracer^{49,50}.

Neuroreceptor imaging during direct agonist and antagonist exposure is also employed to evaluate competitive binding and target engagement. For example, using both a D1 and D2 selective radiotracer, it was shown that a promising antipsychotic drug is a full D1 agonist *in vivo*⁵¹. As a secondary effect, direct agonist and antagonists can also affect autoreceptors, thus changing neurotransmitter levels indirectly. Agonist exposure *in vivo* – both exogenous and endogenous, has also been shown to trigger receptor internalization⁵². Hence, even if exogenous drugs are targeted to directly act at a given receptor, they can influence receptor availability and downstream signaling long-term⁵³.

Neurotransmitter release by drugs—Several exogenous drugs modulate endogenous neurotransmitter levels by either affecting neurotransmitter release or reuptake. Stimulants release very large levels of dopamine, and inhibit reuptake of dopamine, with the net effect that more dopamine stays in the synaptic cleft for a longer period of time. Perhaps the most

commonly investigated stimulant is amphetamine due to its potency. Studies with amphetamine have demonstrated significant decreases (>20% occupancy) in D2/D3 receptor availability in the striatum and in the cortex^{54–57}. It has been shown that these decreases are directly correlated to dopamine release timing and magnitude both in striatal and cortical regions^{58,59}. Yet, such studies have also demonstrated that potent agonists can induce receptor internalization⁵². This can affect the PET imaging signal, such as that of [¹¹C]raclopride, which shows artificially prolonged decrease of radioligand binding after amphetamine exposure⁶⁰.

As opposed to exogenous drugs that can be specific to just one subtype of receptor class, neurotransmitters can bind to all subtypes of their respective receptor, albeit with differential affinity. *In vivo*, the probability of neurotransmitter binding to one subtype of receptor is further determined by the distribution of the concentration of specific subtype receptors in the brain that can differ significantly between anatomical regions⁶¹.

Route of drug administration—The fastest and most effective way of administering a drug is by injecting a liquid form of the drug intravenously (i.v.). Acute i.v. injections of amphetamine have shown that the drug can reach its target within less than a couple of minutes¹. Due to the fast response, this route of administration makes it a preferred method for within-scan challenges that can observe changes in receptor binding in real-time, depending on the radiotracer kinetics.

An alternative method is to administer a drug orally. Usually, the uptake of the drug is on a timeline of several hours, so that the drug is given several hours before the PET imaging scan is acquired. It is important to note that dosing strategies between oral and i.v. administration can be very different. In order to achieve the same receptor occupancy in the brain, usually a much larger dose needs to be given orally compared to i.v. injected amounts.

Finally, neurotransmitter releasing drugs can also be inhaled, such as in the case of nicotine through cigarette smoking^{62–64}. In this context, 2-[¹⁸F]FA has been applied to reveal changes in $\alpha 4\beta 2$ nACh receptor, for example by cigarette smoking showing that that smoking of one cigarette produced almost 90 % receptor occupancy⁶⁵. Another study indicated that the use of electronic cigarettes leads to dose-dependent significant $\beta 2$ -nACh receptor occupancy together with reduction of craving similar with the use of a tobacco cigarette⁶⁶.

Behavioral challenges

There has been a lot of interest in studying endogenous release of neurotransmitters and how they determine behavior and cognition. To this end, behavioral tasks have been specifically designed that are thought to modulate neurotransmitter release. PET imaging is then used to capture dynamic changes using a single injection protocol that includes kinetic modeling approaches to capture the dynamic changes using a single radiotracer injection. Alternatively, an average change from one state to the other is measured using a double bolus injection protocol, where the first injection serves as a baseline for the second study.

Studies with dopaminergic radiotracers—The dopaminergic involvement in reward processing has long been identified with the processing of rewarding stimuli. Dopaminergic neurons have been shown to release dopamine in response to a rewarding stimulus and encode the error in the prediction of reward⁶⁷. Using PET dopaminergic tracers, several studies have investigated this phenomenon using a reward-based task.

The role of striatal dopamine in mediating the responses to reinforcing stimuli has been demonstrated using [¹¹C]raclopride. Koeppe et al.⁶⁸ demonstrated that endogenous dopamine release in response to a behavioral challenge can be detected by [¹¹C]raclopride. They showed that that [¹¹C]raclopride binding potential was reduced by 13% in the ventral striatum in an activation scan, where the first 50 min consisted of a video game with monetary incentives, compared to a separate baseline scan. Striatal dopamine has also been found to be involved in the cognitive planning of a novel action and during the performance of executive processes. Using a Montreal card sorting task, a reduction by up to 13% in [¹¹C]raclopride binding potentials was found in subregions of the caudate and putamen⁶⁹.

While the previous studies all used a double bolus injection, single scan approaches also successfully demonstrated striatal dopamine release through reduced receptor availability in behavioral tasks. A single scan design that uses the first portion of the curve after radiotracer injection for a baseline measurement and then a period of activation at a later point in the same scan has the advantage that it increases statistical power while reducing interscan variability^{70,71}. Studies that investigated working memory demonstrated significant changes in [¹¹C]raclopride binding in the ventral striatum during a positively rewarding task⁷⁰. The use of a finger-tapping motor task also showed decreases in [¹¹C]raclopride receptor availability in subregions of the putamen⁷². Similar decreases in [¹¹C]raclopride receptor binding have also been shown during emotional processing tasks and executive inhibition tasks^{73,74}. Several kinetic modeling approaches have been proposed to accurately analyze such experiments that use a within-scan challenges with just one radiotracer injection^{72,75}. Overall, such methods enable the imaging of dopaminergic neurotransmission in the live human brain⁷⁶.

Cortical dopamine has been suggested to be a key neurotransmitter in the regulation of working memory and has led to a number of studies investigating the release of dopamine during a working memory task. Both the radiotracers [¹¹C]FLB457 and [¹⁸F]fallypride have shown decreased receptor binding in the ventrolateral frontal cortex and left medial temporal lobe during working memory and attention^{77,78}. Decreases in binding by [¹¹C]FLB457 have been shown to be directly related to dopamine release with microdialysis measurements⁵⁹. For a comprehensive review of imaging human cognitive function in the dopaminergic system, see Cropley et al. (2006)⁷⁹.

Neuromodulation techniques

It has been shown that modulation of brain activity through implantable (or external) devices alters neuronal firing as well as neurotransmitter levels. Neuromodulatory treatments through implantable devices like deep brain stimulation electrodes or non-invasive modulation like transcranial magnetic stimulation demonstrate that clinical symptoms can be improved. Imaging signatures of such neuromodulatory interventions have given an insight

into mechanisms behind stimulation, and started to characterize the metabolic and receptor-specific changes that occur during and with long-term stimulation^{80,81}.

Challenge studies with other non-dopaminergic radiotracers

Evidence for PET radiotracer binding being sensitive to changes in endogenous opioid release originated from several clinical non-pharmacological stimulation studies including pain⁸², emotional responses⁸³, and neuromodulation techniques^{84,85}, as well as pharmacologically induced by amphetamine⁸⁶, buprenorphine⁸⁷, and placebo administration⁸⁸ using mostly [¹¹C]carfentanil. Other did find no significant differences in [¹¹C]carfentanil BP_{ND} between amphetamine and placebo conditions⁸⁹ implying that an i.v. dose of amphetamine does not cause acute opioid release in healthy human subjects. Thus, the postulated role of the opioid system in mediating the effects of amphetamine needs to be further investigated. To assess whether such pain-induced changes in opioid neurotransmission contribute a significant component of the blood oxygenation level dependent (BOLD) functional MRI signal, simultaneous PET/MRI has been performed using [¹¹C]diprenorphine and dynamic data acquisition⁹⁰. This study showed co-localized responses in thalamus and striatum related to pain processing indicating a correlation between opioid release and functional hemodynamic activation during pain.

Cholinergic, mACh and nACh receptor (subtype) selective radiotracers have shown sensitivity to acetylcholine in monkeys and humans by significant reductions in PET outcome measures after intravenous administration of physostigmine (a reversible acetylcholine esterase inhibitor)²⁵. Thus, physostigmine infusion leads to significant reductions of 2-[¹⁸F]FA⁹¹, (-)-[¹⁸F]flubatine⁹², and [¹⁸F]nifene binding⁹³. These preclinical investigations suggest that sensitivity for measuring changes of extracellular acetylcholine could be feasible with all these tracers. In keeping with this assumption, a recent study in humans demonstrated the use of bolus/infusion protocol for accurate quantification of (-)-[¹⁸F]flubatine binding parameters in 120 min of physostigmine-induced changes in acetylcholine levels⁹⁴ suitable for future application in clinical studies.

To date, however, no methodology has been sufficiently validated for assessment of acute changes in extracellular serotonin in the human brain. According to a recent review of the data, the most credible combination of tracer and releaser to interact with the 5-HT receptors directly and produce a spurious displacement signal would be [¹¹C]Cimbi-36 (as it is an agonist) and 3,4-methylenedioxy-N-methylamphetamine (MDMA) (as neither it or its metabolites have measurable affinity at the 5-HT_{2A} site)⁹⁵. Evidence that the tracers are sensitive to changes in serotonin concentrations induced by selective serotonin reuptake inhibitors (SSRI) is limited since studies also suggest that single doses of SSRIs do not increase but rather decrease extracellular serotonin concentrations in serotonergic projection regions, as assessed with PET⁹⁶.

III. Multi-modal imaging of receptor functional dynamics with PET and fMRI

Opportunities for multimodal imaging of receptor function with combined PET and fMRI

MRI and PET have become established imaging modalities with a wide range of applications in the clinic and for biomedical research. Especially for the field of neuroimaging, both technologies have advanced diagnosis and treatment through *in vivo* whole-brain imaging techniques. The strengths of MRI lie not only in its high resolution and excellent soft tissue contrast for morphological imaging, but also in imaging physiological parameters such as hemodynamics, diffusion or metabolites. However, MRI is limited in its sensitivity and specificity for biochemical changes. Those features are exactly what PET excels at: Its sensitivity in the picomolar range (10^{-11} to 10^{-12} mol/L) is extraordinary compared to MR sensitivity of around 10^{-3} to 10^{-5} mol/L⁴⁷. Furthermore, PET has very high biochemical specificity, such that quantification of *in vivo* biochemistry is possible. This is one of the reasons why it has become the gold standard for imaging glucose metabolism, with its main clinical application in oncology. Nevertheless, it often lacks anatomical information and is inferior in its spatial and temporal resolution compared to MRI. A full comparison of PET and fMRI measurement capabilities is shown in Table 1. The combination of both MRI and PET thus integrates complementary imaging techniques, allowing for the simultaneous measurement and mapping of specific neurochemistry, functional physiology and its underlying anatomy.

The development of fMRI techniques has advanced behavioral and translational neuroscience by extending traditional anatomical brain imaging to include maps of human brain function. Concurrently, PET has become the gold standard for the imaging of specific *in vivo* neurochemistry. The combination of these two modalities into one scanner opens up a promising new research area with the potential to provide insight into neurochemistry of the brain and to understand the underlying biochemical nature of brain function and its dynamics.

Mapping receptor function with PET and fMRI

Functional MRI measures a hemodynamic response that is secondary to underlying neuronal activation. The metabolism involved in neuronal activity causes a local vascular response that can be detected by fMRI. The notion of “neurovascular coupling” describes the link between a neuronal response and subsequent changes in vascular parameters: At the most basic level, neurotransmitter concentrations and receptor occupancy at the synapse provide the conversion of a chemical signaling link to electrical activity in the neuron. Neuronal activity is then linked to a vascular response through energy consumption and metabolism, in which astrocytes are known to play a significant role.

A relatively unexplored question to date is how neuroreceptor signaling supports the functional signaling or neurovascular coupling chain. Comparing fMRI signal with other techniques that either directly measure neuronal activity or quantify related metabolic or hemodynamic parameters has brought about advances in our understanding of neurovascular coupling mechanisms. Such correlations of fMRI with other non-invasive and invasive measurements have been able to characterize its relationship to neuronal activity^{97–99}.

Below, we discuss recent findings and the importance of PET and fMRI, and its simultaneous acquisition, as complementary tools to investigate the link between neuroreceptor-specific activation and the downstream effects on hemodynamics - a measure of brain function. Figure 1 shows how neuroreceptor PET and fMRI signal modulation can be correlated to advance our understanding of *in vivo* receptor-specific signal modulation at the whole-brain level.

PET/fMRI studies that have directly correlated neuroreceptor occupancy with pharmacologically induced changes in functional signaling demonstrated neurovascular coupling to receptor occupancy have been demonstrated in the receptor system using [¹¹C]raclopride. In a series of studies, the effects of D2/D3 specific agonist and antagonist challenges were tested to characterize receptor function with PET/fMRI in non-human primates: Simultaneous measurement of D2/D3 receptor occupancy and fMRI signal due to D2/D3 blocking or stimulation revealed correlations in anatomical space and with dose^{49,50}. For D2/D3 antagonist binding, a direct neurovascular coupling mechanisms to occupancy was shown in time. These studies directly demonstrated that it is possible to determine functionality of receptors in a dose-response pattern: An inhibitory agonist elicits a decrease in cerebral blood volume (CBV), whereas an antagonist elicits an increase in CBV that is dose-dependent to receptor occupancy. Other efforts to decode the neurovascular coupling between dopamine receptor function and a concomitant hemodynamic change provide a solid basis for the interpretation of dopaminergically related signal. fMRI with drug challenges has consistently shown that D1-like agonism is associated with increased hemodynamics changes, while D2-like agonism is associated with negative hemodynamic changes^{100–103}. Data further show that hemodynamic changes (measured using either BOLD or CBV) are strongly correlated with dopamine release measured using microdialysis at high dopamine levels, and are mediated for opposing changes at low dopamine release levels due to the higher affinity of D2 over D1 receptors^{104–108}. Together, these animal data are surprisingly robust, with numerous studies across different dopamine antagonists and agonists of specific sub-types showing no exceptions to the coupling rules of D1/D5 yielding positive changes and D2/D3 yielding negative changes¹¹.

Human studies that evaluated receptor modulation and functional brain signatures using BOLD have taken several different approaches that have advanced our understanding of receptor-specific brain function. In a study that investigated pain modulation, simultaneously co-localized fMRI and PET signal changes with the opioid-ligand [¹¹C]diprenorphine were found in the thalamus and striatum. The positive correlation between thalamic changes in binding potential and magnitude of BOLD signal change suggests that pain-induced changes in opioid receptor binding directly contributes to the fMRI signal change in this region⁹⁰. Other studies have investigated sequentially obtained PET receptor availability and fMRI signal in the same subjects: In the context of the opioid receptor system, BOLD response patterns due to watching movie clips of painful situations were shown to be correlated with μ -opioid receptor availability, as measured with [¹¹C]carfentanil, but not with D2 receptor availability, as measured with [¹¹C]raclopride¹⁰⁹, suggesting the involvement of the opioid system, but not the dopamine system in vicarious pain. Several investigations of the dopamine system have studied the reward system with the use of a monetary incentive delay task and have demonstrated an *in vivo* coupling between the BOLD response and striatal

dopaminergic neurotransmission with [^{11}C]raclopride^{110,111}. Investigations of the D2 receptor system in patients have shown that schizophrenic patients show a deficit in the ability to release dopamine after amphetamine exposure, and that the amount of dopamine release correlates positively with BOLD signal changes due to a working memory task⁵⁶. Together, these studies have provided *in vivo* insights about the neuromolecular pathways involved in the formation of brain functional processing in healthy humans and psychiatric disease.

Functional connectivity measurements have also been compared to receptor availability at rest or during modulation by behavioral tasks. While performing a task, intrinsic functional networks are thought to direct attentional resources away from internal stimuli and focus on task engagement. The ability to relate receptor-specific parameters to functional network changes could advance our understanding of the molecular underpinnings of network-wide effects. For example, it has been shown that low cortical D1 density predicts stronger decoupling of default mode and frontoparietal control networks during a working memory task¹¹². In human bonding, the medial amygdala network plays a crucial part and may be associated to increased endogenous dopamine responses when mothers watch their own infants¹¹³. In schizophrenia, abnormal striatal functional connectivity patterns can be detected that correlate with lower D2 receptor density in cortical and subcortical brain regions, and are likely affected by a deficiency to release dopamine^{114,115}.

Together, these PET/fMRI studies have already advanced our understanding of the molecular signatures involved in functional networks and neurotransmission. Yet, the robustness of some of these signals, their subregional specificity and involvement of other neurotransmitters still needs to be determined. In some cases, fMRI may be able to validate PET findings or vice versa, and in other cases, PET and fMRI signals may lead to new models hypotheses on receptor-based neurotransmission in a realistic *in vivo* setting.

***In vivo* imaging of the dynamics of neurotransmitters and receptor function**

Receptors in the brain are not static entities, but rather exhibit dynamic behavior at cellular and membrane levels, as well as on temporal scales from seconds up to months or years. Traditional neuroreceptor measurements made using PET have, for most of its history, provided a temporal snapshot of a single point in time. “Dynamic” PET in this setting often refers to the time course of dynamic tracer kinetic uptake and the longer-term temporal scales of hours, days or even years, not the real-time fast dynamics of the receptor systems themselves. The latter requires relatively fast *in vivo* dissociation rates, or a short residence time for radiotracers at their target, while still being specific. In contrast to this, the dynamics of functional imaging with fMRI in this context are driven mainly by the temporal resolution of the fMRI acquisition and are fast compared to PET (see Table 1). Functional changes at the receptor can thus routinely be observed on the order of seconds using dynamic fMRI acquisitions. Imaging such real-time changes simultaneously give insight into real time *in vivo* “receptor dynamics” or “receptor function”. Figure 2 provides an overview of dynamic comparisons for assessing receptor binding and function with both PET and fMRI, and different experimental options for acquiring measurements of dynamic modulation with each modality.

Today, we know that many dynamic receptor-specific changes occur on timescales that are minutes or much less. Neurotransmitter release, receptor trafficking, up- or down-regulation are all dynamic phenomena occurring on timeframes of seconds to minutes. For example, phasic dopamine release, which is connected to cognitive activity and psychostimulants, can be modulated at timescales on the order of seconds^{116–118}. At somewhat longer time scales, tonic changes in dopamine concentrations also occur in response to these stimuli, which typically evolve over time frames of minutes. But while measurements of properties such as receptor internalization or externalization can be made in cell culture, there is a lack of studies and methods to measure the receptor dynamics of *in vivo* intact tissue.

Advances in detecting real-time dynamic changes in binding at receptors with PET have been brought about by pushing both novel experimental designs as well as analysis methods. While the sensitivity of PET is extremely high, the sensitivity of PET to changes in receptor binding, or the dynamic range, is determined and inherently limited by each radiotracer. To increase the sensitivity to changes in receptor availability experimentally for any tracer, bolus plus infusion injections can be used. This has been shown first for [¹¹C]raclopride⁵⁴ and in addition can simplify quantification of radiotracer binding. Infusion injections can even be applied to irreversibly binding radiotracers like [¹⁸F]FDG¹¹⁹. In the latter study, it was shown that dynamic uptake in [¹⁸F]FDG can be detected due to visual stimuli in real-time. In general, the detection of real-time molecular changes requires a within-scan challenge, where an initial baseline dataset is acquired at the beginning of the scan and after an appropriate timeframe (which is radiotracer-dependent) an intervention or stimulus is started. Together, such experimental designs enable the observation of short-time dynamic changes.

For accurate quantification of dynamic PET signals, the development and use of appropriate dynamic kinetic analysis models are of equal importance. Towards this goal, models that include a dynamic association rate as part of the kinetic model have been developed^{72,75}. To date, these models have been successfully applied for the detection of dopamine release or exogenous D2 receptor binding using the radiotracer [¹¹C]raclopride for evaluating cigarette smoking^{62,120} or drug injections⁵⁰. The applicability and validation for the use of such methods for other radiotracers remains to be investigated in more detail.

The combination of dynamic receptor occupancy studies using PET, along with rapid physiological measures of neurovascular coupling with MRI, promises to create a more in-depth understanding of receptor dynamics. It has already been shown that PET and fMRI signals can be modeled quantitatively to map receptor internalization rates *in vivo*⁵⁰. The combination of fMRI measures, with their rapid temporal responsiveness, together with dynamic and quantitative measurements of receptor occupancy using PET as described above, can provide a unique framework for developing quantitative assays of dynamic dopamine release and dopamine receptor and transporter trafficking in near real time.

Overall, to advance and make use of the full potential of the combination of PET and fMRI, it is important for our methods to be able to both be responsive to very rapid neurotransmitter release, and have stability over substantially longer time frames in order to be able to assess both neurotransmitter and receptor functional dynamics in real time.

Future outlook on neurotransmitter imaging with PET and fMRI

PET and fMRI have each brought about their own advances in *in vivo* imaging of neurotransmission. PET receptor imaging has enabled detailed studies of specific receptor classes and established sensitivity for endogenous neurotransmitter release for some of the radiotracers at time resolutions that are relevant for acute stimuli as well as long-term changes. The use of fMRI due to targeted pharmacological stimuli and in specific behavioral settings has established functionally relevant neurovascular coupling mechanisms that can be directly attributed to neurotransmitter release and receptor binding, and allows the interpretation of functional signaling of receptors in real time. With the introduction of simultaneous PET/fMRI systems, progress has been made towards multiparametric imaging that pushes beyond the knowledge generated by either one modality. The parallel measurement and interpretation of hemodynamics, functional connectivity and changes in neurotransmitter levels across the entire brain and in near real time promises to give new insights into receptor function and their networks in the living brain.

While PET neuroreceptor radioligands are designed to be highly specific, fMRI signal is sensitive to any ligand or receptor class involved in neurotransmission. Simultaneous measurements of PET and fMRI can thus provide specificity while capturing the summed response of a receptor to a stimulus. The power of combining these two measurements lies in being able to identify the separate inputs that lead to a functional response. The timescales that are relevant to measure detectable signals on both PET and fMRI simultaneously have already proven to be feasible within one dynamic scan session^{50,119}. Yet, improved sensitivity through experimental design, modeling of acquired data, advanced scanner hardware and fast radiotracer kinetics will all contribute towards deciphering the complexity of dynamic receptor signaling over different timing windows.

Quantification and combined modeling of PET and fMRI signals will be needed to understand the mechanisms behind observed signal changes. Receptors or their downstream response can be directly further modulated if there are already other ligands present on the same target, also known as cooperative binding. A sigmoidal binding curve is used to describe the fraction of the receptor molecule saturated by the ligand as a function of the ligand concentration in this case. To estimate the number of ligand molecules that are required to bind to a receptor to produce a functional effect, classes of different subreceptors and receptors with more than one ligand binding site have to be considered. Such assumptions on multi-site binding and changes in receptor transformation produce a variety of ligand dose-response curve phenotypes more appropriately suited to modeling ligand-receptor interactions. There is a need to quantify receptors of different properties across the brain, regional differences in fractional receptor occupancy as well as the detection of small changes in binding due to neurotransmitter release². To find the appropriate, probably multi-ligand receptor models from these receptor occupancy studies represents the major challenge.

PET/fMRI will likely help to estimate functional drug responses at the receptor level *in vivo* together with the potency of a drug (e.g. dopamine stabilizers) in addition to receptor occupancy. In particular, if there are seemingly non-dose proportional clinical effects and drugs acting on different receptors, and *in vivo* evaluation tool with PET/fMRI will be

extremely valuable. Such studies in the living human brain are of unmet need since *in vitro* data might not necessarily reflect the true functional levels or might differ by orders of magnitude in their affinities from what we see *in vivo*. An extended quantitative description of drug properties *in vivo* including tissue exposure, target engagement, and pharmacological activity¹²¹, molecular neuroimaging with simultaneous PET/fMRI will increasingly be part of the central nervous system drug-development process (from preclinical studies to clinical trials), thereby reducing sample sizes, associated costs and risks of these studies.

In clinical research studies, neuroreceptor PET/MRI will play an important role to help decipher the mechanisms underlying brain dysfunction, and aid in the diagnosis and personalized therapy planning. An assessment with PET/fMRI for optimal drug dosing strategies could be crucial when trying to avoid side effects that often appear at high occupancies that are too high. Being able to characterize the nuances in functional effects due to exposure of different drugs with PET/fMRI may lead to a better understanding of both on- and off-target effects and more targeted and personalized treatment plans. Finally, investigating the functional effects of neurotransmitter overload or deficiency in psychiatric disease, or understanding the underlying receptor-specific signatures in disrupted networks will contribute to a more holistic understanding of brain function and dysfunction in health and disease.

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Figure 1.

Overview and examples of receptor binding studies using neuroreceptor PET or functional MRI (fMRI). While PET can provide insight into neurotransmitter or drug binding at specific receptor systems, fMRI can measure a functional response related to the same receptor and stimulus. The example shows dose-dependent D2/D3 receptor binding of an agonist with PET and correlated fMRI responses due to D2/D3 agonist and antagonist action. (Displayed images are modified with permission from previously published work by Sander et al.^{49,50}). Overall, various types of PET receptor and fMRI measurements can be compared to understand correlations or discrepancies in functional signals.

Figure 2.

Overview of dynamic signal acquisitions with PET and fMRI. PET time-activity curves (TACs) can be modulated through a variety of radiotracer and challenge administrations. fMRI signal changes can also be observed through different types of experimental design. Determining the best design for simultaneous dynamic PET and fMRI signal modulation in each context is an important step in understanding the dynamic relationship between neuroreceptor PET and fMRI signature.

Table 1

Comparison of PET and fMRI techniques as tools for imaging neurotransmission. PET excels in molecular sensitivity and specificity to its target, while fMRI has excellent temporal resolution and can have higher sensitivity to signal modulation. At receptors, PET can give a readout for receptor occupancy and fMRI can provide a measure of receptor function.

	Neuroreceptor PET	Functional MRI
Spatial resolution	1.5 – 12 mm	0.1 – 3 mm
Temporal resolution	0.5 min – 10 min	0.5 – 5 seconds
Observable dynamic changes	~10 min (radiotracer-dependent)	0.5 – 10 seconds
Molecular sensitivity to target	10^{-11} – 10^{-12} M	10^{-3} – 10^{-5} M
Detectable signal changes from baseline	~ 10 % (radiotracer-dependent)	~ 1 % (study- and acquisition-dependent)
Pharmacokinetics/dynamics	Yes, for radiotracer	Not directly
Receptor occupancy	Yes	No
Receptor function	No	Yes
Tracer level doses	Yes	Dependent on acquisition. (endogenous contrast: BOLD, ASL)