Molecular Imaging of the CNS: Drug Actions

Thomas Mueggler and Markus Rudin

Introduction: Neuroreceptor and Functional Imaging

Imaging has always played a major role in the diagnosis and staging of CNS disorders and in the evaluation of therapeutic interventions. While in the past imaging applications were focused on the structural, physiological, and metabolic characterization of CNS pathologies, in the last two decades methods have emerged that enable to noninvasively study the neurochemistry of the living brain. These methods, predominantly based on nuclear imaging modalities such as positron emission tomography (PET) and single photon emission computed tomography (SPECT), use radioligands targeting specific neuroreceptors. The ever-increasing availability of radiotracers for neuroactive compounds (neurotransmitter synthesis/metabolism, enzymes, transporters, receptors, neuromodulators, and second messengers) provides an attractive toolset both for basic neuroscience, diagnosis and patient care. Complementary to such specific molecular (PET) probes in diagnosis of diseases and patient management, generic tracers can be used to derive physiological and/or metabolic information. [^{18}F]-2-fluoro-2-deoxyglucsoe (FDG) PET provides information on the regional cerebral metabolic rate of glucose ($rCMR_{glu}$) (Phelps et al. 1979), while [^{15}O]-H_2O can be used to assess regional cerebral blood flood flow (rCBF) have been widely applied to assess brain activity. Alternatively, brain function can be assessed using functional MRI (fMRI), which measures hemodynamic changes elicited through the neurovascular coupling (Ogawa et al. 1992). Both PET and fMRI methods have emerged to become standard techniques for the clinical evaluation of drug candidates providing an indirect readout (via hemodynamic coupling) of the drug effects on CNS activity. This topic has been the subject of many reviews.

M. Rudin (✉)
Institute for Biomedical Engineering, University and ETH Zürich, HCI D426,
Wolfgang-Pauli-Strasse 10, CH-8093, Zürich, Switzerland
e-mail: rudin@biomed.ee.ethz.ch

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In this chapter we will focus on the applications of molecular (PET) imaging probes to characterize specific CNS disorders, to evaluate the outcome of therapeutic interventions in neuropsychiatric disorders such as mood, anxiety, and psychotic disorders (categories according to the Diagnostic and Statistical Manual of Mental Disorders, 4th Edition). In the second part we will discuss current attempts to use molecular imaging for diagnosing and staging (diagnose) of neurodegenerative disorders such as Parkinson's (PD) and Alzheimer's disease (AD) and for evaluating the outcome of treatment.

Application of Neuroreceptor and Functional Imaging to Psychiatric Disorders

Major Depression and Bipolar Affective Disorder

Contemporary models of mood disorders, i.e., major depression disorder (MDD) and bipolar affective disorder (BD), emphasize that mood symptoms arise from disruptions in the interactions of limbic/paralimbic neural networks involved in emotional and cognitive processing. The understanding of how the neurotransmitter systems within those affected neuroanatomical structures are involved together with the modulatory role of glucocorticoids, inflammatory cytokines, and brain-derived growth factors led to an integrated model of depression (Maletic et al. 2007). Symptoms of mood disorders are viewed as a result of disturbances in the ability to regulate emotions, attention, and memory supported by these networks under normal conditions. Mayberg's limbic-cortical model of depression involves dorsal, ventral, and rostral compartments, each of which accounts for some portion of the constellation or symptoms associated with depression (Mayberg 1997). Consistent with this model resting state PET and SPECT studies in patients with MDD and BD have consistently found decreased frontal lobe function as reflected by decreased regional cerebral glucose metabolism (rCMRglu) and decreased rCBF in the dorsal compartments of the prefrontal cortex and the cingulate. Increases in rCMRglu and rCBF have been observed in the ventral compartment including orbitofrontal and sugenunal prefrontal cortex, amygdala, and insular cortex. Increases in rCMRglc and rCBF in the limbic/paralimbic system also include the anterior temporal cortex. In addition, alterations in basal ganglia and thalamic function have been reported. These imaging read-outs indicative of aberrant energy turnover have been complemented by information on the integrity of specific neurotransmitter systems. In fact, the dominant hypothesis guiding research and treatment in MDD and of depressed phases in BD is that of decreased monoaminergic function: norepinephrine, dopamine and, in particular, serotonin (Fuller and Wong 1990; Vermetten and Bremner 2002a, b; Nutt 2008) as reflected by the modern classes of antidepressants being, the selective serotonin reuptake inhibitors (SSRIs), the serotonin and norepinephrine reuptake inhibitors (SNRIs), norepinephrine reuptake

inhibitors (NET), and the norepinephrine-dopamine reuptake inhibitor (NDRI) bupropion. Several specific molecular (PET/SPECT) ligands to target monoaminergic function have been developed and are used for the management of patients suffering from MDD and BD.

The evidence supporting the role of serotonin (5-hydroxytryptamine, 5-HT) dysfunction in MDD includes studies of neurochemical markers in post mortem brain of the neuroendocrine response to acute pharmacologic interventions of the serotonin system and of pharmacologic manipulations of serotonin systems and their effects on mood (as reviewed by (Kalia et al. 2005). Progress in medical chemistry of drugs interfering with the serotonergic system has promoted also the development of imaging agents for PET and SPECT. Besides the alpha-[^{11}C]-methyl-l-tryptophan as surrogate marker of the cerebral 5-HT synthetic rate, most of the available selective or nonselective radiotracers to date target 5-HT$_{1A}$ or 5-HT$_2$ receptors or the serotonin re-uptake transporter (SERT) (Fig. 1). SERT is

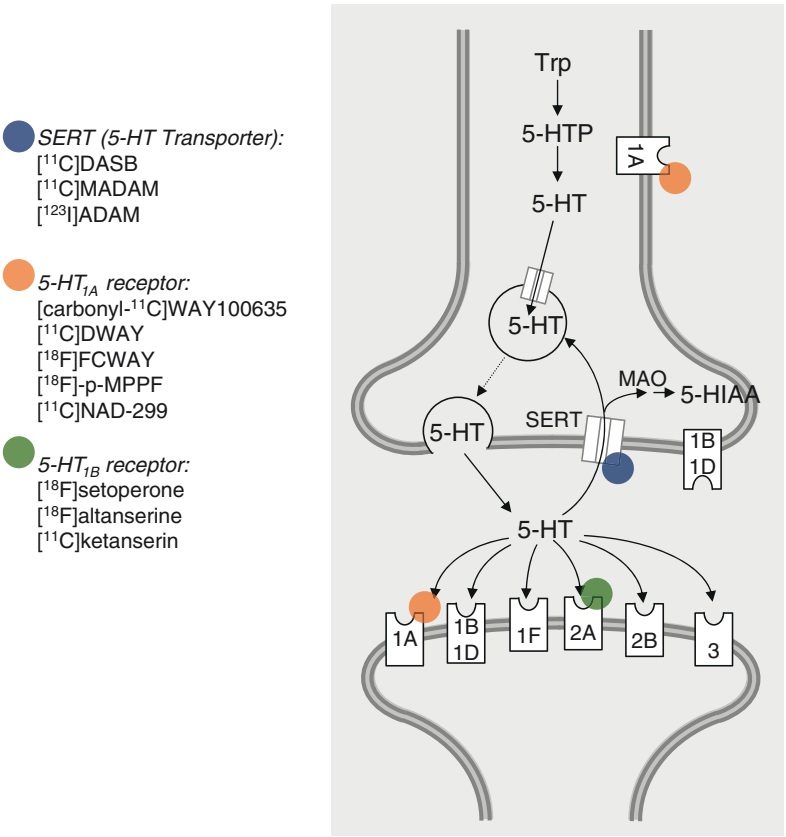

Fig. 1 Available radiotracers targeting 5-HT transporter, 5-HT$_{1A}$ and 5-HT$_{1A}$ receptors

the primary target for selective serotonin reuptake inhibitors (SSRIs) used in treatment of mood and anxiety disorders. SSRIs bind to the transporter thereby blocking the reuptake of 5-HT which raises extracellular serotonin levels and stimulating a cascade of intracellular events including the desensitization of 5-HT_{1A} autoreceptors. This leads to an increase in neuronal firing rate and to downstream trophic effects that are correlated with the clinical response (Blier and de Montigny 1999; Duman et al. 1997). Multiple attempts have been made to synthesize suitable radiolabeled tracers targeting SERT; mostly, clinically approved SSRIs have been labeled with the positron-emitting isotopes, ^{11}C or ^{18}F, and their value as PET tracer evaluated in primates. Currently, two structurally related PET radiotracers suitable for imaging SERT levels in the human brain are at hand: [^{11}C]*N*,*N*-dimethyl-2-2-amino-4-cyanophenylthiobenzylamine ([^{11}C]DASB) (Frankle et al. 2006; Kim et al. 2006; Wilson et al. 2002; Houle et al. 2000) and [^{11}C]-*N*,*N*-dimethyl-2-(2-amino-4-methylphenylthio)benzylamine ([^{11}C]MADAM) (Lundberg et al. 2006; Halldin et al. 2005; Larsen et al. 2004). In parallel, [^{123}I]-2-((2-((dimethylamino)methyl)phenyl)thio)-5-iodophenylamine ([^{123}I]-ADAM) has been reported to be a suitable tracer for SPECT imaging of SERT in humans (Catafau et al. 2005; Kauppinen et al. 2003). Most of the 5-HT_{1A} receptor antagonist radioligands which have been evaluated for PET imaging (Pike et al. 2000; Cliffe 2000) bear structural similarity to the 5-HT_{1A} antagonist, *N*-[2-[4-(2-methoxyphenyl)-1-piperazinyl]ethyl]-*N*-(2-pyridinyl)-cyclohexane carboxamide (WAY100635). Among them the [carbonyl-^{11}C]WAY100635 (Hall et al. 1997), [^{11}C]DWAY (Pike et al. 1998) and their fluorinated analogs [^{18}F]FCWAY (Lang et al. 2006) and [^{18}F]-p-MPPF (Shiue et al. 1997) appear to be the most useful PET ligands for the quantification of 5-HT_{1A} receptors in humans (Kumar et al. 2007). Robalzotan (NAD-299), a structurally different compound, has been labeled with carbon-11, and preliminary results show that [^{11}C]NAD-299 is a promising imaging probe for the 5-HT_{1A} receptors (Sandell et al. 2002). 5-HT2_{A} receptor antagonism is a property of certain antipsychotic and antidepressant drugs (Moresco et al. 2006; Bockaert et al. 2006). Specific radioligands for PET such as [^{18}F]setoperone (Blin et al. 1988), [^{18}F]altanserine (Lemaire et al. 1991), ^{11}C-ketanserin (Baron et al. 1985), and for SPECT ([^{123}I]5-I-R91150) (Peremans et al. 2003) are available allowing to investigate in vivo the exact role of the 5-HT_{2A} receptor availability in the time course of symptoms of depressive, mood, or eating disorders (Moresco et al. 2006; Moeller et al. 2006), and borderline personality disorder (Soloff et al. 2007). A selective 5-HT_{2A} receptor antagonist MDL-100907 [(2,3-dimethoxy-phenyl)-{1-[2-(4-fluorophenyl)-ethyl}-piperidin-4-yl}-methanol] has been introduced (Lundkvist et al. 1996) and validated in a number of studies in human and nonhuman primates (for review see Moresco et al. 2006).

In vivo imaging studies on neuroreceptor expression levels have confirmed the hypothesis of decreased 5-HT neurotransmission in depressed patients. In an early study, Agren et al. (1991) reported reduced uptake of [^{11}C]-5-hydroxy-tryptophan in depressed patients. Further investigations on the 5-HT transporter or 5-HT receptor have only recently been possible as a result of major advances

in ligand development. The majority of these studies have been conducted in midlife depressed patients. The current study is to assess the binding potential (BP) of SERT an index of the transporter density and affinity. PET/SPECT studies comprising a number of different SERT ligands, among them the state-of-the-art [^{11}C] DASB, have been reviewed by Meyer et al. It can be concluded from these imaging readouts that the contributing mechanism to extracellular serotonin loss is excessive 5-HTT rather than loss of serotonin neurons through degenerative processes. Imaging studies reporting on regional decreases in 5-HTT BP include other comorbid axis I illnesses (presence of a substance use, anxiety, or eating disorder) and disorders in their sampling. This reflects effects of common comorbid illnesses rather than MDD alone, whereas a significantly greater 5-HTT BP can been found in MDD subjects with more severe pessimism (Meyer et al. 2007). A further important aspect is receptor (i.e., transporter) occupancy. Endogenous displacement of [^{11}C]DASB may occur on the 5-HTT with large magnitude changes in extracellular 5-HT but would not be expected to occur with extracellular 5-HT changes that are physiologically relevant for humans. Studies using [^{11}C]DASB carried out in the rhesus monkey, the cat, and the rat brain showed decreased 5-HTT BP in several brain regions after a pharmacologically induced increase in the interstitial serotonin (5-HT) concentration. In vivo binding of [^{11}C]DASP was studied before and after having increased interstitial 5-HT concentrations using tranylcypromine (TCP), which inhibits the enzyme (monoamine oxidase, MAO). The critical point here is that the rise in extracellular serotonin with high doses of tranylcypromine is enormous, with a several hundred to thousand percent rise being typical. This magnitude of serotonin change may exceed what is physiologically relevant in humans. In fact the effect of tryptophan depletion upon 5-HTT has shown no effect, demonstrating that endogenous serotonin occupancy is unlikely to appreciably influence [^{11}C]DASB43 under physiologically tolerable conditions (Meyer et al. 2007).

The investigation of serotonin transporter occupancy by SSRIs using SERT PET ligands has revealed a high degree of SERT occupancy at relatively low plasma concentrations of SSRIs paroxetine, citalopram (Meyer et al. 2001, 2004; Voineskos et al. 2007), clomipramine, and fluvoxamine (Suhara et al. 2003) venlafaxine, and sertraline (Voineskos et al. 2007). It could be inferred that 80% occupancy of the 5-HTT is a necessary condition for demonstrating successful SSRI treatment for depressive episodes in clinical trials and, therefore, has a relationship to the clinical effect. However, the exact relationship between SERT occupancy and clinical efficacy of SSRIs has not yet been evaluated.

The development of 5-HT$_{1A}$ R-selective positron emission tomography (PET) radioligands has enabled in vivo measures of presynaptic and postsynaptic 5-HT$_{1A}$ R binding in depression. Drevets et al. demonstrated that the mean 5-HT$_{1A}$ R-binding potential (BP) was reduced in the mesiotemporal cortex (MTC) and raphe in unmedicated depressives relative to controls using PET and [carbonyl-^{11}C]WAY-100635 (Drevets et al. 1999). Similar reductions were evident in the parietal and medial occipital/posterior cingulate cortices. These data were consistent with those of Sargent et al. (2000), who found decreased 5-HT1AR BP,

measured using PET and [^{11}C]WAY-100635, in unmedicated depressives relative to controls in the raphe, MTC, insula, anterior cingulate cortex, temporal polar cortex, and orbital cortex. Similarly, Meltzer et al. (2004) found significantly decreased [^{11}C]WAY-100635 binding in the raphe in elderly depressed patients relative to age-matched controls. Several studies reported that these reductions in 5-HT$_{1A}$ R binding also were evident in currently remitted patients with a history of MDD (Sargent et al. 2000; Moses-Kolko et al. 2007; Bhagwagar et al. 2004), suggesting that this abnormality persists across illness episodes in recurrent depression.

An important property of 5-HT$_2$ receptors is that their receptor density has an inverse relation to extracellular serotonin levels, such that the density of 5-HT2 receptors in the cortex increases after chronic serotonin depletion and decreases after chronically raising extracellular serotonin (Meyer et al. 2007). To investigate to what degree a reduction in 5-HT$_{2A}$ receptor availability can be used to predict the clinical efficacy of antidepressant agents several PET studies in patients suffering from MDD have been reported. However, the measured changes in the 5-HT$_{2A}$ receptor availability during the course of antidepressant therapy (i.e., SSRI) are contradictory (Attar-Levy et al. 1999; Meyer et al. 2001; Mischoulon et al. 2002). Based on a binding study using the nonselective 5-HT$_2$ receptor PET ligand [^{18}F] fluoro-ethyl-spiperone (FESP) (Coenen et al. 1988) in patients classified according to their clinical response and treated with paroxetine it has been stated that the 5-HT$_2$ receptor behaves more as a state marker (presence or absence of symptoms) than a trait marker (presence of MDD) (Moresco et al. 2006).

The physiological actions of DA in the brain mediate functions that are commonly disturbed in MDD, such as reward, motivation, initiation of movement, and cognitive functions (Nestler and Carlezon 2006) implicating the involvement of dopamine in the pathophysiology of MDD, and many antidepressant drugs have shown to increase dopamine D$_2$ and D$_3$ receptor (D$_2$R/D$_3$R) density and affinity specifically in the mesolimbic dopamine system. Radionuclear Imaging has been used to study the involvement of striatal dopamine in the etiology of MDD (for review see Hirvonen et al. 2008) using [^{11}C]- or [^{18}F]-DOPA to assess presynaptic DA turnover, the SPECT DA transporter ligands [^{99m}Tc]TRODAT-1 and [^{123}I]β-CIT or the D$_2$R ligands [^{11}C]raclopride and [^{123}I]IBZM for PET and SPECT, respectively (Fig. 2). The assessment of striatal D$_2$R density is of interest, considering that this index can be used to indirectly estimate changes in the synaptic concentration of dopamine (Laruelle 2000) and the postsynaptic responsivity of the nigrostriatal dopamine system. These studies have shown either higher (Shah et al. 1997; Meyer et al. 2006), lower (Montgomery et al. 2007), or unchanged (Parsey et al. 2001; Kuroda et al. 2006) striatal D$_2$ receptor density in MDD as compared with controls. However, most of the studies have been performed in patients who have a history of antidepressant pharmacotherapy. Higher D$_2$ receptor density in the striatum has also been found in postmortem brain tissue of MDD diagnosed and medicated suicide victims but not in medication-free MDD patients (Bowden et al. 1997). Many imaging studies have recruited and analyzed patients with a specific subtype of

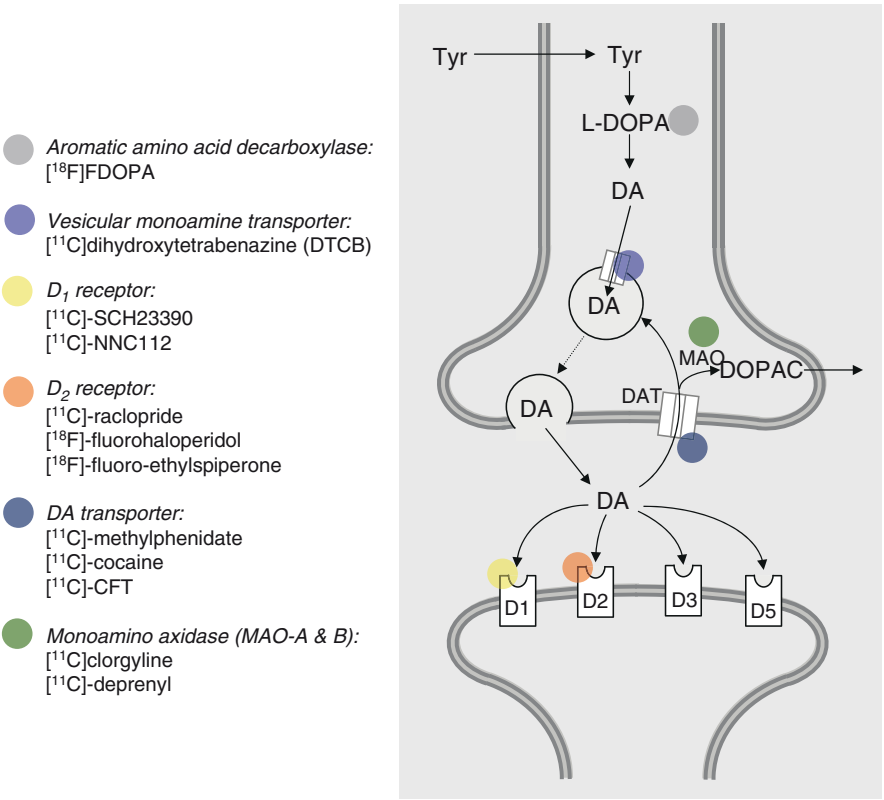

Fig. 2 Selective and nonselective PET radiotracers targeting the dopaminergic system

MDD (such as those with motor retardation or the melancholic subtype). Thus the results may not be generalizable to the population of depressed patients as a whole. In a recent PET study using [^{11}C]raclopride Hirvonen et al. (2008) reported striatal *and* thalamic D_2 receptor-binding values in treatment-seeking drug-free patients MDD patients and healthy controls. In all regions analyzed no statistically significant differences in [^{11}C] raclopride BP were observed between the two groups. Correlation analysis of depressive symptoms severity vs. [^{11}C]raclopride BP showed no association in any region. Unaltered thalamic D_2 receptor binding in MDD is consistent with the results of Montgomery et al. (2007) which show no differences between MDD patients and controls using the high affinity $D_{2/3}$ receptor ligand [^{11}C]FLB-457. However, the lack of altered dopamine D_2 receptor density in the sample patients is *not* in disagreement with the dopamine hypothesis in antidepressant effect. According to this hypothesis, the sensitivity of the postsynaptic dopamine receptor system is increased, specifically in the mesolimbic dopamine system targeting the nucleus accumbens (Gershon et al. 2007). It should be noted

that if the affinity of D_2/D_3 receptors is altered in MDD even before antidepressant treatment, it cannot be detected using antagonist radioligands such as [^{11}C]raclopride (which bind with equal affinity to receptor configured in low- and high-affinity states). A difference could only be observed with receptor agonist tracers that prefer the high-affinity state (Finnema et al. 2005; Hwang et al. 2005; Willeit et al. 2006).

Selective NE reuptake inhibitors (NRIs) (e.g., reboxetin) have found use for the treatment of depression, and many antidepressants have substantial potential occupancy of the NET at recommended dosages or are combined as SNRIs [i.e., duloxetine; Gupta et al. 2007). Given that the NE transporter (NET) is also a binding site for cocaine and drugs of abuse, there is a great need for a probe to assess the densities of NET in vivo by brain imaging with either positron emission tomography (PET) or single photon emission tomography (SPECT). However, despite the importance of understanding this transporter's role in psychiatric disease and treatment, a suitable radioligand for studying NET has been slow to emerge. Several potent NET-selective antidepressants, among them nisoxetine, oxaprotiline, lortalamine, and analogs of reboxetine, have been labeled for in vitro or in vivo mapping of brain NET (Ding et al. 2005; Kung et al. 2004; Schou et al. 2003; Seneca et al. 2006; Kanegawa et al. 2006; Tamagnan et al. 2007). Unfortunately, most of these candidate ligands do show a high in vivo nonspecific binding excluding their utility as NET imaging agents. So far only the (S,S)-reboxetine-based agents such as *O*-methyl ((S,S)-[^{11}C]-MeNER) (Schou et al. 2003), *O*-fluoromethyl ((S,S)-[18F] FMeNERD2) (Seneca et al. 2006), and 2-iodo ((S,S)-[123I]IPBM) (Kanegawa et al. 2006; Tamagnan et al. 2007) analogs showed many desired in vivo properties for imaging. In an attempt to further optimize (S,S) isomers of the reboxetine motif (i.e., shorter time-to-peak equilibrium) new structures and new analogs have been synthesized and their in-vitro-binding affinities measured (Zeng et al. 2008). Results of comparative microPET studies of these tracers in nonhuman primate brain are needed to estimate the potential of theses radiotracers for their application in clinical studies.

Anxiety Disorders

Pathological anxiety is classified into five types: obsessive-compulsive disorder (OCD), phobias, panic disorder, post-traumatic stress disorder (PTSD), and generalized anxiety disorder. Notably, a large proportion of psychiatric disorders comprise pathological anxiety as a comorbidity which can for example in the case of mood disorder condition demonstrate a considerable overlap of clinical symptoms and pathophysiological processes. The neurobiological basis of anxiety has been reviewed extensively (Vermetten and Bremner 2002a, b; Kent et al. 2002; Ressler and Nemeroff 2000). For example, the role of the benzodiazepine (BZD) receptor has been studied in patients suffering from panic disorders and post-traumatic stress

disorder (PTSD). Neuroimaging studies in panic disorder patients using either the SPECT ligand [^{123}I]-iomazenil or the PET ligand [^{11}C]-flumazenil have revealed decreased BZD receptor binding relative to normal controls. In particular, [^{123}I]-iomazenil binding was found to be decreased in the left hippocampus and precuneus of the patients (medication free for six weeks and BZD free for four weeks prior to scanning) binding was higher than control values in the right caudate, cuneus, right middle frontal gyrus, and left middle temporal gyrus. In patients who experienced a panic attack during scanning binding decreased throughout all brain structures when compared to patients with no panic attack. Besides the BZD receptor system attention has been drawn on the 5-HT$_{1A}$. Using [^{11}C]WAY-100635 PET a significant relationship has been reported between 5-HT$_{1A}$ BP and anxiety (Tauscher et al. 2001)

Psychotic Disorders

Schizophrenia is a mental disorder associated with abnormal perception or expression of reality (hallucinations, paranoia, disorganized speech, and thinking). From a neurochemical perspective, a critical role is attributed to dysfunctional DA neurotransmission involving the mesolimbic system. The importance of DA becomes obvious as drug-induced inhibition of DA function was found to reduce psychotic symptoms. Hence it was hypothesized that excessive DA neurotransmission was associated with the positive symptoms of schizophrenia. Today, the efficacy of atypical neuroleptics, which interfere less with the DA system but also affect 5-HT, has demonstrated that DA dysfunction alone does not account for the disorder (Jones and Pilowsky 2002). In addition, abnormal low levels of glutamate receptors in the brains of deceased schizophrenia patients implied an important role of glutamate and impaired function of NMDA receptors (Konradi and Heckers 2003). In addition it was shown that inhibition of NMDA receptors may cause psychosis-like behavior (Lahti et al. 2001), as a severe side effect that has prevented the development of NMDA inhibitor drug for other CNS indications, e.g., acute cerebral ischemia.

One of the best examples documenting the value of receptor imaging refers to treatment of schizophrenia patients with D_2R inhibitors. In line with the increased activity of the DA system, it has been found that D_2R densities are upregulated in the brains of approximately 70% of schizophrenia patients (Verhoeff 1999). First-line antipsychotic therapy is therefore aimed at inhibiting D_2R-induced signaling. In order to achieve therapeutic efficacy, a significant percentage of D_2R has to be occupied by the inhibitor. Yet initial studies revealed that there is a narrow range between therapeutic doses and doses causing severe adverse motor side effects (extrapyramidal side effects) (Halldin et al. 1998). PET studies using the D_2R ligand [^{11}C]raclopride in schizophrenic patients treated with the D_2R antagonist haloperidol revealed that a minimal striatal receptor occupancy (RO) of 60% is

required to produce an optimal antipsychotic response, while receptor occupancies exceeding 75–80% prompted side effects (Verhoeff 1999). Clearly the availability of a D_2R PET ligand has made a significant contribution to the clinical management of schizophrenia patients. [^{11}C]raclopride is reproducible and reliable (Hirvonen et al. 2003), and this paradigm has been used to show altered thalamic D_2 receptor binding in patients with schizophrenia (Talvik et al. 2006).

In addition to its important clinical role, it may represent an attractive tool for basic biological research and in particular for drug discovery and development. For example, [^{11}C]-raclopride in combination with microPET imaging has been used to study D_2R-binding characteristics in rats. It has been found that D_2R receptor binding can be studied reproducibly in a quantitative manner. Injections of 9.25 MBq of [^{11}C]-raclopride (approximately 250 μCi) provided sufficient sensitivity for quantitative determination of the distribution volume ratio (Alexoff et al. 2003). Animal PET using this radiotracer constitutes an attractive approach to study D_2R-ligand-induced modulation of dopaminergic transmission, enabling the determination of the temporo-spatial distribution of candidate drugs, the assessment of their receptor occupancy, and the relationship between receptor interaction and downstream (therapeutic) efficacy. The relative high activity of radiotracer that is commonly used in these studies due to sensitivity reasons constitutes a potential confound. Hence in the data analysis it should be considered that the concentration of the tracer is not necessarily much smaller than that of the drug candidate (Alexoff et al. 2003). The sensitivity of the method was evaluated in comparing D_2R binding of [^{11}C]-raclopride in normal wild-type and D_2R knock-out (KO) mice. KO mice showed significantly lower binding in the strital reaclopride binding than wild-type animals. The striatal-to-cerebellar activity ratio was 1.33 ± 0.13 for wild-type mice and 1.05 ± 0.03 for KO mice (Thanos et al. 2002).

A very different application of PET imaging of D_2R binding is to use it as reporter system for studying gene expression in vivo as demonstrated, for example, in adenoviral delivery systems and in tumor xenografts. Overexpression of dopamine D_2R in the rat striatum through gene transfer mediated through an adenoviral vector led to significantly higher local activity of D_2R PET ligands [^{11}C]-raclopride, [^{11}C]-nemonapride, and [^{11}C]*N*-methylspiperone, when compared to the contralateral striatum, which was injected with a control vector. Coinjection with an excess of "cold" raclopride inhibited the binding of [^{11}C]-raclopride. On the other hand, the uptake of neither a D_1R-specific nor a DAT-specific PET ligand was not different between the two striata demonstrating the specificity of the readout (Ogawa et al. 2000). An important characteristic of using D_2R as a reporter gene is to uncouple the ligand receptor from potential downstream effects, e.g., the modulation of cyclic AMP levels. This can be achieved through mutations: it has been demonstrated using various assays including PET with the ligand 3-(2'-[^{18}F]-fluoroethyl)-spiperone (FESP) that the D2R80A mutant has still the full capability as a PET reporter gene, i.e., efficiently binding the radioligand FESP, while it does not modulate cAMP levels following ligand binding (Liang et al. 2001).

Application of Neuroreceptor and Molecular Imaging to Neurodegenerative Disorders

Alzheimer's Disease

Diagnosis and Imaging Targets

Early detection of AD is imperative for studying the pathophysiological mechanisms leading to disease and for efficient clinical treatment of this disorder. The characteristic signature of AD is the deposition of amyloid-β plaques and neurofibrillary tangles (NFTs) in the patient's brain, which parallels the disease progression, but can only be diagnosed with certainty by autopsy. Key pathological features of AD – senile plaques (SPs) and NFTs (Hardy and Selkoe et al. 2002) – are identified using either histopathological dyes such as Congo Red (CR) or Thioflavin T (ThT) or by immunohistochemistry. SPs and NFTs are mainly composed of aggregated (polymeric) forms of amyloid-β (Aβ) peptide (composed of either 40 or 42 amino acids) and hyperphosphorylated Tau (phospho-Tau) proteins, respectively (Glenner and Wong 1984). Beta amyloid (Aβ 1–42) in its fibrillar form (Hardy and Selkoe et al. 2002) or as soluble oligomers (Selkoe 2002) has been suggested as the primary cause of AD. It is still an open question whether soluble or insoluble oligomers or mature amyloids are more toxic. The amount of soluble Aβ in brain seems to correlate better with impairment of cognition than do plaque counts (Näslund et al. 2000). Other potentially concomitant processes especially tau phosphorylation and downstream events such as oxidative stress, inflammatory reactions, microglia activations, play a crucial role in the AD pathology (Mattson 2004). It was also recently suggested that tangles may precede amyloid plaques (Schoenheit et al. 2004). The ultimate effects of complex inflammatory, ionic, and oxidative changes that occur in affected brain regions are neuritic dystrophy, as well as selective synaptic and neuronal loss. Presumably, these processes occur gradually over many years in the preclinical asymptomatic phase of AD, which may involve early synaptic dysfunction, and then continue during its clinical progression.

Imaging of Energy Turnover

First and second generation of imaging biomarkers included mainly altered glucose metabolism (assessed by [^{18}F]FDG-PET) as surrogate of synaptic dysfunction and [^{15}O]PET for rCBF deficits along with general and/or region-specific structural abnormalities as predictor of gross neuronal atrophy. PET studies have thereby been performed under baseline conditions to assess resting state metabolic activity, as well as during the performance of various cognitive tasks. Using FDG-PET the largest reduction in resting state $CGMR_{glu}$ has been found in the posterior cingulate cortex (Minoshima et al. 1997). Despite some promising results, it was soon realized from these clinical imaging studies that patterns of brain atrophy and

hypometabolism offered only limited diagnostic specificity for differentiating AD form other types of dementia; these nonspecific imaging readouts are therefore of limited value as surrogate markers of early disease, i.e., for mild-to-moderate stages of AD.

PET Imaging of Cholinergic Neurotransmission: Radioligands for Muscarinic and Nicotinic Acetylcholine Receptors and Acetylcholine Esterase

An obvious result of the synaptotoxicity is loss of cholinergic enzymes (choline acetyltransferase and acetylcholinesterase AChE) leading to massive deficits in the cholinergic system (Davies and Maloney 1976; Whitehouse et al. 1981) followed by an impairment of other neurotransmitter and neuromodulator systems. AchE blockers have been shown to be beneficial as symptomatic treatment in AD. Thus in vivo imaging of AChE activity might yield immediate insight into the drug-induced modulation of cholinergic function. Not surprisingly, a significant number of imaging studies conducted thus far in AD patients have focused on evaluating the hypothesis of cholinergic hypofunctionality in AD.

A considerable number of imaging agents targeting the cholinergic system in vivo have been developed. This includes radiotracers developed for measurements of the vesicular acetylcholine transporter ([^{123}I]-iodovescamicol; Kuhl et al. 1994), AChE activity such as *N*-[^{11}C]methylpiperidin-4-yl propionate ([^{11}C]-PMP (Koeppe et al. 1999), [^{11}C]-physostigmine (Pappata et al. 1996), as well as ligands for the acetylcholine receptors (AChR = The AchR of the muscarinic type (mAChR) can be assessed using PET and SPECT labels such the C11 labeled ligands [^{11}C]-benztropine (Dewey et al. 1990), [^{11}C]-scopolamine (Frey et al. 1992), [^{11}C]-*N*-methyl-4-piperidylbenzilate [^{11}C]-NMPB) (Suhara et al. 1993), the F18 labeled muscarinic agonist, 3-(3-(3-[^{18}F]Flouropropyl)thio)-1,2,5-thiadiazol-4-yl)-1,2,5,6-tetrahydro-1-methylpyridine ([^{18}]F-TZTP/[^{18}]FP-FTZP) (Kiesewetter et al. 1995; Podruchny et al. 2003), or the iodinated compounds 3-quinuclidinyl 4-[^{123}I]-iodobenzylate [^{123}I]-QNB (Eckelman et al. 1984), [^{123}I]-4-iododexetimide, and [^{123}I]4-iodolevetimide (Muller-Gartner et al. 1992). With the exception of the radiotracer [^{18}F]FP-FTZP that is selective for the M2 muscarinic receptor subtypes, the other muscarinic radiotracers are not subtype specific (Eckelman 2001). For the AchR of the nicotinergic type (nAChR) ligands have been developed such as [^{11}C]-nicotine (Nordberg et al. 1995) or [^{18}F]fluoro-A-85380 (Horti et al. 2000). It has to be mentioned that the cholinergic receptors and therefore radiotracers have a widespread distribution in the cortex, subcortical, and limbic structures consistent with the distribution observed in post mortem autoradiographic studies in human brain. As a result, it is difficult to identify a region that is devoid of AChE or of the receptors to use as a nonspecific binding reference, rendering the quantitative analysis more difficult.

These PET ligands have been extensively used for diagnostic purposes and in particular for demonstrating pharmacological proof of principle of cholinergic drugs, which is of clinical relevance as AChE inhibitors are currently the most commonly used class of medications to treat cognitive and behavioral symptoms in AD. Several studies have also evaluated a potential implication of the monoamine systems in

AD or the use of radiotracers for the DA system to eventually distinguish Lewy body dementia (DLB) from AD.

The involvement of nAChRs in vivo has so far been studied by analyzing nicotine binding. In an earlier PET study, it was demonstrated that [^{11}C]-nicotine binding was lower in the brains of AD patients compared with that in control subjects, reflecting a loss in high- and low-affinity nAChRsites (Nordberg et al. 1990). In addition, significantly lower levels of [^{11}C]-nicotine binding have been observed in the frontal and the temporal cortex, and hippocampus of patients with AD compared with controls (Nordberg et al. 1995). After treatment with AChE inhibitors such as tacrine, an increase in [^{11}C]-nicotine binding has been found in cortical regions in patients with AD after short-term (3 months) treatment (Nordberg et al. 1997). A few AChE inhibitors such as galantamine and physostigmine are known to interact with nAChRs as allosterically potentiating ligands. Their efficacy is due to slowing down of the receptor desensitization as well as by sensitizing the nAChRs, which increases the probability of channel opening induced by acetylcholine or by nicotinic agonists (Maelicke et al. 2000).

Imaging of Abeta

Despite the relevance of the cholinergic system in AD and the importance of imaging cholinergic transmission in AD patient care, such studies are only indirectly related to the pathophysiological processes leading to AD. Therefore significant efforts have been undertaken to develop noninvasive imaging tools that are directly probing molecular species in AD pathophysiology, e.g., to assess the Aβ plaque load in vivo. It is without saying that such methods would be of tremendous benefit for tracking disease progression and for monitoring therapy's efficacy in clinical trials. So far four such radioligands, out of a significantly larger number of tracer candidates, have been advanced toward clinical evaluation using cohorts of AD patients: [^{11}C] PIB (Klunk et al. 2004), [^{18}F]FDDNP (Shoghi-Jadid et al. 2002), and [^{11}C]SB13 (Verhoeff et al. 2004), and [^{11}C]BF-227 (Kudo et al. 2006). All four ligands display uptake and retention in areas of the brain that are known to contain high densities of plaques (for review see Furumoto et al. 2007; Nordberg et al. 2004). At present, the tracer with most supporting in vivo data is [^{11}C]PIB.

Parkinson's Disease

Diagnosis and Imaging Targets

Parkinson's disease (PD) is a progressive multifocal central nervous system (CNS) degenerative disease. Clinical features of PD patients are associated with degeneration of dopaminergic nigrostriatal neurons; they include the typical motor symptoms of bradykinesia, rigidity, and tremor. In advanced disease stages, additional symptoms

emerge, which result from degeneration of nondopaminergic as well as dopaminergic pathways. The clinical onset of motor dysfunction related to PD is linked to a depletion of DA levels by 60–80%, which corresponds to the death of approximately 30–40% of the neurons in the substantia nigra pars compacta (SNc). A further histopathological hallmark of the disease is the presence of intracytoplasmic inclusions called Lewy bodies in the remaining dopaminergic neurons of the substantia nigra. These eosinophilic aggregates are predominantly composed of aggregated forms of the protein α-synuclein (Goetz et al. 2006), which is normally located presynaptically.

Common pharmacological therapies are mainly based on increasing cerebral DA levels by administration of the precursor L-DOPA (levodopa, L-3,4-dihydroxyphenylalanin), which is taken up through the blood-brain barrier and converted to DA through decarboxylation by the aromatic amino acid decarboxylase (AAAD). L-DOPA treatment as such is limited to relieving the symptoms of PD without modulating the course of the disease.

Imaging in PD

PD is associated with reduced neurotransmission through the dopaminergic system, which might arise either from reduced dopamine availability or due to impaired dopamine sensitivity at the postsynaptic neuron or both. It is obvious that the PET probes targeting the molecular players of the DA system together with functional readouts of brain activity such as FDG-PET and fMRI play an important role both for diagnostics and for guiding therapy regimens in these patients. With the availability of radioligands to monitor both presynaptic and postsynaptic DA function (Mazière et al. 1992) noninvasive imaging of PD has largely been in the domain of positron-emission tomography (PET) and single-positron-emission tomography (SPECT) imaging in rat models. These PET studies have been complemented by pharmacological fMRI experiments that assessed the neural activity induced by administration of L-DOPA (Jenkins et al. 2002), the D_1R and D_2R agonists apomorphine (Zhang et al. 2001), amphetamine or a dopamine transporter agonists (Chen et al. 1997).

Many of the PET probes to test various aspects of the dopaminergic system have been already discussed (see the section on schizophrenia). In view of the clear correlation of low DA levels with the severity of PD symptoms, a noninvasive readout of the local DA synthesis rate would be clinically relevant. DA synthesis rate can be estimated from PET studies using a labeled DA precursor, [^{18}F]-fluoroDOPA ([^{18}F]-FDOPA) (Firnau et al. 1987). Cerebral tracer activity depends on the uptake kinetics and on the activity of the AAAD. As discussed earlier, receptor density at the postsynaptic neuron can be assessed using radiolabeled receptor ligands such as the D_2R ligand [^{11}C]-raclopride. Other aspects of the system can be studied using radiolabels; the design template has been borrowed from compounds with known pharmacological activity. For example, cocaine is known to inhibit reuptake of DA via DAT. Hence, [^{11}C]-labeled cocaine derivatives could be used as PET probes to study the density and binding capacity of DAT. The benzoquinolizine compound

3,4-dihydrotetrabenazine (DTBZ), which has been shown to deplete cerebral monoamines in rat brain by reversibly inhibiting vesicular monoamine transporter 2 (VMAT2) (Pettibone et al. 1984), was originally used as an antipsychotic drug and more recently to treat hyperkinesia disorders. Correspondingly, the labeled [^{11}C]-DTBZ might serve as a tool to probe VMAT2 distribution (Goswami et al. 2006). Similarly, [^{11}C]-labeled clorgyline enables measuring of the MAO-A activity, an enzyme involved in monoamine degradation. Finally, in vivo imaging of rodents can be used to characterize the chronic effects of drug treatments using a single animal, thus mimicking long-term drug therapy in humans. For example, PET imaging using [^{11}C]raclopride of the rat brain has been used to help elucidate a neurochemical basis for fluctuations in the efficacy of chronic L-dopa treatment of Parkinson's disease (Opacka-Juffry et al. 1998; Hume et al. 1995).

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