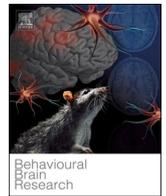




Contents lists available at ScienceDirect

Behavioural Brain Research

journal homepage: www.elsevier.com/locate/bbr

Molecular imaging of schizophrenia: Neurochemical findings in a heterogeneous and evolving disorder

Paul Cumming^{a,b,*}, Anissa Abi-Dargham^c, Gerhard Gründer^d

^a Department of Nuclear Medicine, Inselspital, Bern University, Bern, Switzerland

^b School of Psychology and Counselling, Queensland University of Technology, Brisbane, Australia

^c Stony Brook University, Renaissance School of Medicine, Stony Brook, New York, USA

^d Central Institute of Mental Health, Department of Molecular Neuroimaging, Medical Faculty Mannheim, University of Heidelberg, Mannheim, Germany

ARTICLE INFO

Keywords:

PET
SPECT
Molecular imaging
Schizophrenia
Dopamine

ABSTRACT

The past four decades have seen enormous efforts placed on a search for molecular markers of schizophrenia using positron emission tomography (PET) and single photon emission computed tomography (SPECT). In this narrative review, we cast a broad net to define and summarize what researchers have learned about schizophrenia from molecular imaging studies. Some PET studies of brain energy metabolism with the glucose analogue FDG have shown a hypofrontality defect in patients with schizophrenia, but more generally indicate a loss of metabolic coherence between different brain regions. An early finding of significantly increased striatal trapping of the dopamine synthesis tracer FDOPA has survived a meta-analysis of many replications, but the increase is not pathognomonic of the disorder, since one half of patients have entirely normal dopamine synthesis capacity. Similarly, competition SPECT studies show greater basal and amphetamine-evoked dopamine occupancy at post-synaptic dopamine D_{2/3} receptors in patients with schizophrenia, but the difference is likewise not pathognomonic. We thus propose that molecular imaging studies of brain dopamine indicate neurochemical heterogeneity within the diagnostic entity of schizophrenia. Occupancy studies have established the relevant target engagement by antipsychotic medications at dopamine D_{2/3} receptors in living brain. There is evidence for elevated frontal cortical dopamine D₁ receptors, especially in relation to cognitive deficits in schizophrenia. There is a general lack of consistent findings of abnormalities in serotonin markers, but some evidence for decreased levels of nicotinic receptors in patients. There are sparse and somewhat inconsistent findings of reduced binding of muscarinic, glutamate, and opioid receptors ligands, inconsistent findings of microglial activation, and very recently, evidence of globally reduced levels of synaptic proteins in brain of patients. One study reports a decline in histone acetylase binding that is confined to the dorsolateral prefrontal cortex. In most contexts, the phase of the disease and effects of past or present medication can obscure or confound PET and SPECT findings in schizophrenia.

1. Introduction

Emil Kraepelin, in his clinical description of a progressive psychiatric disorder, popularized the term *dementia praecox*, which dated back to earlier work by Bénédict Augustin Morel and Arnold Pick. With the increasing rejection of Kraepelin's diagnostic dichotomy of *dementia praecox* versus manic-depressive psychosis, Paul Eugen Bleuler introduced the term *schizophrenias* to denote his clinical description of a splitting between the emotional and the intellectual functions of the personality, and considered dementia to be a secondary aspect of a disorder of unknown physical basis. To this day, clinicians and

researchers generally concur that the clusters of positive and negative symptoms of schizophrenia and associated cognitive deficits are manifestations of an organic brain disease. However, a century of research has not revealed any pathogen, nor does any single genetic factor carry great weight in the incidence of schizophrenia, so the underlying or causal neuropathology remains elusive. Following upon early findings of ventricular enlargement to pneumoencephalography, there has been a huge expenditure of efforts in carrying out structural magnetic resonance imaging (MRI) in schizophrenia patients. Meta-analyses of many such studies consistently show ventricular enlargement and cortical volume loss, especially in the temporal lobe and its associated structures

* Corresponding author.

E-mail address: paul.cumming@insel.ch (P. Cumming).

<https://doi.org/10.1016/j.bbr.2020.113004>

Received 16 September 2020; Received in revised form 22 October 2020; Accepted 31 October 2020

Available online 13 November 2020

0166-4328/© 2020 The Authors. Published by Elsevier B.V. This is an open access article under the CC BY license (<http://creativecommons.org/licenses/by/4.0/>).

such as hippocampus and amygdala [1,2]. A wide range of MR spectroscopy studies has indicated abnormalities in brain glutamatergic metabolism, redox balance (NAD⁺/NADH ratio), and mitochondrial metabolism in patients with schizophrenia [3]. While such findings certainly suggest that something is amiss with cerebral metabolism of patients with schizophrenia, we focus this review on findings from molecular imaging studies with positron emission tomography (PET) and single photon emission computer tomography (SPET/SPECT) that examine specific aspects of metabolism and neurotransmission in schizophrenia. Rationalized pharmacological therapy for schizophrenia should properly derive from knowledge of its neurochemical pathology, as in the case of levodopa treatment of Parkinson's disease, but, as shall be seen, the case is less clear for schizophrenia.

The objective of the present review is to summarize the results of four decades of molecular imaging research in schizophrenia. What then have almost two generations of molecular imaging research taught us about the pathology of schizophrenia, and how might this growing knowledge eventually inform a rational and individualized treatment of this dreadful disease? Towards answering these questions, we have undertaken a non-systematic literature search in the PubMed database with key words SPET/SPECT + schizophrenia (which yielded 654 hits) and PET + schizophrenia (which yielded 1277 hits). Passing quickly over the 75 SPECT studies of cerebral blood flow in schizophrenia, and making a selection of representative neuroenergetics PET studies with [¹⁸F]fluorodeoxyglucose (FDG), we screened out the remaining literature on PET and SPECT investigations of specific neurochemical markers in patients with schizophrenia. Indeed, the search for a neurochemical defect in schizophrenia motivated decades of research into plasma and urinary metabolites from patients, long preceding the advent of molecular brain imaging. Here, the history of the pink spot is a cautionary tale. Chromatographic analysis of urine extracts from patients revealed a pink spot that was absent from samples from control subjects. This spot was tentatively identified as a metabolite of mescaline [4], which seemed to fit with ideas about abnormal biogenic monoamine metabolism that were current at the time. However, later work called into question the pink spot's chemical identity and specificity as a marker in schizophrenia [5]. After a flurry of research, the pink spot proved to be a catecholamine metabolite present in tea, which was commonly drunk to excess by in-patients [6]. Improved technology yields new spots, in a manner of speaking; a recent FDG-PET case study of cerebral energy metabolism showed intense focal activation in the bilateral locus coeruleus of a patient with schizophrenia [7]. The authors interpreted their finding to reveal metabolic activation of the noradrenaline neurons of the locus coeruleus, due to some factor such as stress, opioid withdrawal, or an idiosyncratic specific aspect of that patient's illness.

2. Cerebral metabolism

The topic of FDG-PET is a good point of departure for a critical review of molecular brain imaging in schizophrenia, being one of the first tracers used for this purpose. FDG is a structural analogue of natural glucose that traces the initial stage of glucose metabolism in living cells. Once injected into the circulation, FDG enters the brain by facilitated diffusion across the blood-brain barrier, and then returns to circulation or is phosphorylated by the enzyme hexokinase in living brain cells, both neurons and glia. Whereas natural glucose phosphate would continue through the glycolytic pathway to pyruvate, and ultimately enter the Krebs cycle, FDG-phosphate proceeds no further along the pathway, as it is not a substrate for the isomerase reaction. Consequently, the accumulation over time of FDG-phosphate formed in living cells gives an index of the local rate of glucose consumption. This rate is synonymous with the total energy consumption of the brain tissue, which is exclusively dependent upon glucose as an energy substrate, except for example under ketogenic conditions.

Given the above consideration, maps of cerebral glucose metabolism from FDG PET studies should potentially reveal metabolic abnormalities

in the brain of patients with schizophrenia. Indeed, an early study tested just this hypothesis, showing a reduction in the relative FDG uptake in medial prefrontal cortex of unmedicated patients [8]. Notably, the individual uptake in frontal cortex of the patients did not show the usual correlation with performance of an auditory discrimination task that is evident in healthy volunteers. Here, the implication is that regional glucose metabolism is perturbed, having lost its normal associations with performance of specific tasks. The findings of hypofrontality to FDG-PET concurred with the frequent observations of lower cerebral perfusion in frontal cortex first seen in xenon-133 inhalation SPECT studies, although with the caveat that hypoperfusion tends to be discovered only while patients perform a cognitive task engaging their frontal cortex [9]. However, a meta-analysis of many studies of perfusion and metabolism showed an overall pattern of hypofrontality not just during task performance, but also during rest, whatever may constitute rest when one is lying in a scanner [10]. The distinction is important; if hypofrontality were only present due to failure to engage in a cognitive task, this would qualify as a state defect, but the occurrence of reduced frontal perfusion and metabolism at rest indicates a trait defect in schizophrenia.

The metabolic disturbances of schizophrenia might emerge from a failure of normal functional connectivity of different brain regions, i.e. thalamo-cortico-striatal circuits, which could manifest in impaired or perhaps errant metabolic coupling between different regions of these circuits. Indeed, a very early test in a group of patients with schizophrenia of the correlational mapping approach showed a near absence of the normal pattern of metabolic coupling under a condition of stimulation with mild electrical shocks [11]. A more recent inter-regional correlation analysis of FDG in a group of never-medicated patients with schizophrenia showed weaker positive correlations between the frontal lobe with other brain regions in the patient group [12]. In a group of 70 medication-free patients, FDG uptake in frontal cortex and thalamus had a negative correlation with total scores in the BPRS, and both with positive and negative subscores [13]. Furthermore, that study showed lower right > left metabolic asymmetry in the patients compared to a group of 30 healthy controls. In a relatively small group of patients, the negative symptomology subscores correlated inversely with FDG uptake, which tended to be especially low in structures of the right hemisphere [14]. This observation harkens back to thoughts about cerebral asymmetry in schizophrenia, derived from Pierre Flor-Henry's observation of an association between schizophreniform psychoses and left temporal lobe epilepsy [15]. *Post mortem* neurochemical analyses have given some fragmentary evidence for neurochemical asymmetry, i.e. higher dopamine levels in the left amygdala of patients with schizophrenia [16]. We shall return to this theme in the review of dopamine PET below.

While the left hemisphere is normally dominant with respect of language function, the hemodynamic response during performance of a motor planning task is transiently greater in the right hemisphere of healthy volunteers [17]. However, a task dependent activation favoring the right side was absent in a group of schizophrenia patients monitored by near infrared spectroscopy [18]. An FDG-PET study performed during a continuous performance task showed higher factor scores for the left temporal lobe in schizophrenia patients in normal controls, where the factor score refers to the weighting of some region towards accounting for the total variance in the measurement [19]. One might suppose that the right hemisphere of schizophrenia patients "stands down" in the face of an attentional task, presumably yielding this function to the left hemisphere.

Higher resolution PET instrumentation enables the investigation of metabolism in smaller anatomic divisions. Thus, a well-powered FDG-PET study in unmedicated schizophrenia patients showed metabolic disturbances in divisions of the thalamus, and reported their clinical correlates [20]. Lower relative metabolism in the pulvinar nucleus had an association with positive symptoms and hallucinations, whereas lower metabolism in the mediodorsal thalamus had an association with

negative symptoms. Notably, the pulvinar's role in mediating reciprocal connections between parietal association cortex and occipital cortex may be consistent with an involvement in positive symptoms. The mediodorsal thalamus has reciprocal connectivity with the prefrontal and anterior cingulate cortex and the amygdala, and receives input from the basal ganglia, and is functionally involved in cognition and learning [21], which may suggest an involvement in negative symptoms of schizophrenia.

3. Presynaptic dopamine markers

Molecular imaging research in schizophrenia placed early emphasis on examinations of brain dopamine. This was due to the long-standing prevalence of a dopamine model of schizophrenia, positing that some disease symptoms are due to a functional excess of brain dopamine. However, the fortuitous availability of effective dopamine tracers at an early stage of molecular imaging research also encouraged that research focus. One of the first such radiopharmaceuticals was the dopamine synthesis tracer 6- ^{18}F fluoro-*L*-DOPA (FDOPA), which accumulates in the basal ganglia in PET studies of healthy volunteers, but not in patients with nigrostriatal degeneration due to acquired parkinsonism [22]. The common carrier for large neutral amino acids reversibly transfers circulating FDOPA across the blood brain barrier. Once in brain, FDOPA is a substrate for the enzyme DOPA decarboxylase, found most abundantly in striatal terminals arising from the midbrain dopamine neurons, where the enzymatic product ^{18}F fluorodopamine is retained for a time within synaptic vesicles. As such, the kinetic model for describing FDOPA kinetics follows closely that described above for FDG, but with the complication that FDOPA also yields a radioactive brain-penetrating metabolite, O-methyl-FDOPA [23]. This must somehow be subtracted from the brain signal if one is to isolate the progressive and specific labeling of dopamine neurons in living brain [24]. The metabolic trapping of ^{18}F fluorodopamine in living brain is imperfect due to its deamination to acidic metabolites, which are free to diffuse from brain, thus leading to progressive loss of specific signal [25]. However, the signal loss is of small magnitude during PET recordings of duration less than 60 min, such that applying an irreversible trapping model results in little bias in the estimation of FDOPA trapping [26].

Applying just such an irreversible binding model (analogous to FDG-PET), the first PET study of presynaptic dopamine synthesis in unmedicated patients with schizophrenia revealed significantly higher striatal trapping compared to that in healthy controls [27]. The same study also reported increased FDOPA uptake in a small group of epilepsy patients with interictal psychosis, suggesting a common dopaminergic component in the two forms of psychosis. This result seemed so important to the construct of a dopaminergic abnormality in schizophrenia that there were numerous replications using FDOPA and other amino acid-based PET tracers for dopamine synthesis capacity. A meta-analysis compiling such results in hundreds of patients and controls showed unambiguously that dopamine synthesis capacity is elevated in patients with schizophrenia (Effect size 0.79, $p < 0.001$) [28]. Indeed, increased dopamine synthesis capacity remains one of the best-attested molecular imaging findings in schizophrenia. The human basal ganglia does not operate in isolation, but is tightly coupled to functioning of the cerebral cortex. Thus, it is notable that the increased FDOPA utilization reported in a small group of patients with schizophrenia correlated inversely with the perfusion increase in prefrontal cortex during performance of an executive function task [29]. In effect, the elevated capacity for dopamine signaling in the basal ganglia seemed to link with disabling or blocking of a cognitive function mediated by the frontal cortex. However, we emphasize that elevated dopamine synthesis capacity is not a pathognomonic sign of schizophrenia, since about half of unmedicated patients have entirely normal FDOPA PET findings. Furthermore, a recent study in unmedicated schizophrenia patients did not show any elevation in striatal dopamine synthesis capacity, perhaps reflecting aspects of reference tissue methods for quantitation [30]. Nonetheless,

that study revealed anatomically distinct associations between FDOPA trapping with reward prediction error signaling to fMRI; unmedicated patients failed to show the normal positive association between dopamine synthesis capacity in limbic striatum with reward prediction error signaling, although FDOPA uptake in associative striatum did correlate with higher scores for positive symptoms.

Less well attested is our report of two-fold increased turnover of the ^{18}F fluorodopamine formed in brain of a group of schizophrenia patients, which we detected by applying an extended compartmental model to prolonged dynamic FDOPA recordings. We interpreted this result to indicate that brain dopamine is "churning" in schizophrenia, with the enhanced dopamine breakdown resulting in a paradoxical state of "poverty in the midst of plenty" [31]. In such a scenario, dopamine signaling might be impaired despite a superabundance of newly synthesized dopamine. Indeed, something similar occurs among healthy volunteers treated with a low dose of an antipsychotic medication, which acutely results in disinhibition of dopamine synthesis via autoreceptor blockade, as revealed by increased FDOPA trapping in striatum [32]. Paradoxically, we saw reduced FDOPA trapping in schizophrenia patients treated sub-chronically with antipsychotic medication [33]. We interpreted this result to be a molecular imaging marker of "depolarization block", an electrophysiological phenomenon when dopamine neurons fall silent in rats that are treated with antipsychotic medication for several weeks [34]. A more recent report seemingly recapitulates our result, showing relatively reduced FDOPA uptake in patients under stable antipsychotic treatment [35], which we argued was a late consequence of prolonged autoreceptor blockade [36]. On the other hand, a new PET study from London failed to show any significant effect of antipsychotic treatment for five weeks on striatal FDOPA utilization in first episode patients [37]. Both studies used roughly similar methods for FDOPA quantitation (linear graphical analysis relative to a reference region), so the disparate results may be a matter of differences in the phase of illness between patient groups, or other factors [38].

There is general concordance in the literature that there is increased dopamine synthesis capacity in populations of unmedicated patients with schizophrenia. A PET study in individuals at ultra-high risk (UHR) for developing schizophrenia showed elevated striatal FDOPA trapping specifically in those patients destined to undergo transition to psychosis [39]. Replication in a second cohort also showed increased FDOPA utilization in UHR individuals [40], and in another study involving 47 UHR cases, retrospective reporting of having experienced childhood adversity (a significant stressor) was associated with higher FDOPA uptake [41]. In addition, there was increased FDOPA uptake in healthy first-degree relatives (siblings and children) of patients with schizophrenia [42]. While of moderate effect sizes, scatter plots of data from these studies all showed significant overlap in results between patients and controls, as is usually the case in the schizophrenia literature. As noted above, a pre-existing difference in the dopamine system revealed by FDOPA PET is not a pathognomonic sign of schizophrenia, since half of patients have normal FDOPA results. Something is clearly amiss with the dopamine system in patients at a group level, but FDOPA PET does not itself offer reliable confirmatory diagnosis on an individual basis. On the other hand, an FDOPA study of groups of treatment-resistant patients and responders showed elevated FDOPA uptake while on medication, especially among the responders [43]. The magnitude of the difference was large, and the authors interpreted their findings as evidence that high/excessive dopamine synthesis capacity was a precondition for good response to antipsychotic treatment. In a continuation of that investigation, the authors reported that non-responders to first line treatment showed lower FDOPA uptake when under treatment with the "second-generation" antipsychotic (SGA) medications clozapine or risperidone [44]. However, since the scans were all recorded while the patients were under treatment with an antipsychotic medication, a contrary interpretation seems possible, i.e. only those responding with a downregulation of dopamine synthesis capacity upon first-line treatment enjoyed full clinical benefits, while those with a refractory

dopamine system had a lesser therapeutic response. A study of longitudinal design with follow-up scanning might distinguish whether low FDOPA uptake in the second-line treatment group was an underlying trait, or a response to SGA medication. Recently, machine-learning techniques applied to baseline FDOPA PET scans in unmedicated patients proved fit to predict their response to first-line antipsychotic treatment with a receiver operating curve area under the curve approaching 0.90 [45]. As such, that may be the first molecular imaging study specifically designed to inform treatment decisions in patients with schizophrenia.

Retention of FDOPA-derived PET signal in nigrostriatal nerve fibres requires its enzymatic transformation to [^{18}F]fluorodopamine, which is stored in synaptic vesicles via the vesicular monoamine transporter (VMAT), or is released as a false neurotransmitter. [^{18}F]Fluorodopamine, like endogenous dopamine, is a substrate for dopamine transporters (DAT) in the plasma membrane, which can transfer it along with dopamine back into the presynaptic terminal. Thus, VMAT and DAT present alternate targets for molecular imaging studies of the dopamine system. There does not seem to have been any PET study of VMAT in patients with schizophrenia. A PET study with the DAT ligand [^{11}C]PE2I found elevated binding potential (BP_{ND} , the specific binding relative to a reference region) in the putamen, midbrain, and thalamus in a group of 21 patients with chronic schizophrenia, and showed that individual results correlated with certain clinical symptoms [46]. However, meta-analysis of 16 PET and SPECT studies comprising groups of nearly 300 patients and controls did not detect any significant difference in DAT availability [47]. The variance of the DAT endpoints was significantly higher in the patient group, thus hinting at heterogeneity of DAT expression, which might reflect effects of previous treatment. Multi-tracer PET studies in non-human primate show that DAT expression has a much higher age-dependence than is typically seen for FDOPA or VMAT [48], suggesting a greater degree of plasticity for DAT. Rather than being simply a passive actor in synaptic transmission, the activity of DAT is a moderator of antipsychotic efficacy; preclinical research has shown that individual rats with upregulated DAT expression in the course of chronic antipsychotic treatment acquire resistance to the behavioral effects of the medication [49,50]. On the other hand, downregulation of DAT activity due to normal aging or in the context of psychostimulant abuse disables dopamine re-uptake from the extracellular space and reduces the efficacy of antipsychotic medication [49,50]. Thus, we suppose that treatment effects, age, and trait heterogeneity all may influence the results of DAT molecular imaging studies in schizophrenia.

4. Post-synaptic dopamine markers

While FDOPA PET and DAT studies give indices of the biochemical integrity of presynaptic dopamine terminals, the dopamine-responsive elements in brain mainly reside on post-synaptic membranes, i.e. on the medium spiny neurons of the striatum. The dopamine receptors are typical G-protein coupled receptors belonging to two broad pharmacological classes, namely the D_1 -like and D_2 -like receptors. Molecular imaging studies have long placed main emphasis on the dopamine D_2 -like receptors, given the antipsychotic properties of many D_2 -antagonists. The very first such studies of schizophrenia used the butyrophenone ligand [^{11}C]NSMP, despite its lack of absolute pharmacological specificity for dopamine receptors and despite its languid kinetics *in vivo*, which present problems for quantitative analysis. Although its equilibrium binding to D_2 -like receptors (the composite of D_2 , D_3 , and the sparse D_4 subtypes) occurs very late in the scan, the initial uptake into brain follows an irreversible binding model. Applying this model to data recorded during the first 60 min after injection, the pioneering study of its type indicated two-fold elevated rate constant (k_3 ; min^{-1}) for the association of [^{11}C]NSMP to its binding sites in the caudate-putamen of drug naïve patients with schizophrenia [51]. That study was eventually followed by an attempt at replication, which showed no group

difference in striatal [^{11}C]NSMP binding between never-medicated patients and healthy volunteers [52].

This discrepancy might have been due to the somewhat problematic quantitation of [^{11}C]NSMP binding. Thus, there was a need for a reversibly binding D_2 -like ligand for molecular imaging studies, a need that was eventually met by the benzamide $\text{D}_{2/3}$ antagonist [^{11}C]raclopride for PET studies and by its chemical congener [^{123}I]IBZM for scintigraphy and SPECT imaging. The benzamide class of molecules has nearly complete pharmacological specificity for dopamine $\text{D}_{2/3}$ receptors, unlike the butyrophenones, which also bind to dopamine D_4 and serotonin $5\text{HT}_{2\text{A}}$ receptors. [^{11}C]Raclopride also has rapid kinetics, approaching equilibrium binding in brain within less than an hour, which facilitates quantitation of its binding potential (BP_{ND}) relative to uptake in a non-binding region such as cerebellum.

The BP_{ND} for a receptor ligand of high molar activity is proportional to the ratio of the saturation binding parameters, B_{max}/K_D , in living brain. However, if the injected mass is so high that there is substantial (>5%) occupancy at the binding sites, then the measured BP_{ND} declines in proportion to that occupancy. Further, if the experimenter measures BP_{ND} twice in the same individual, once with tracer of high molar activity, and again with high injected mass, it becomes possible to separate the parameters B_{max} and K_D , by making a “two-point” Scatchard analysis. This rather onerous experimental design has been successfully applied in a [^{11}C]raclopride PET study of 20 healthy volunteers and 20 unmedicated patients with schizophrenia [53], which showed no group difference either in B_{max} or K_D . In this approach, the B_{max} estimate should be isolated from effects of competition from endogenous dopamine, but the prevailing dopamine concentration *in vivo* can alter the apparent K_D . That neither parameter should differ between groups, would thus argue against important differences in $\text{D}_{2/3}$ receptor occupancy by endogenous dopamine, although experiments described below indicate elevated dopamine binding in patients with schizophrenia.

The various discrepancies in early investigations of striatal dopamine D_2 -like receptor availability in schizophrenia raised a controversy that eventually yielded to a meta-analysis of many PET studies, as well as SPECT studies with the benzamide antagonist ligand [^{123}I]IBZM. A meta-analysis focusing on studies of extrastriatal $\text{D}_{2/3}$ receptors showed small reductions in cortex and thalamus of patients, but this effect did not reach significance and had a confound due to medication history [54]. Compilation of the available molecular imaging studies targeting striatum indicated slightly elevated dopamine $\text{D}_{2/3}$ receptor availability among patients, which was partially attributable to results in older patients, and to studies using butyrophenone ligands [55]. On the other hand, a later meta-analysis of 22 PET/SPECT studies showed higher striatal binding with a summary effect size of 0.26, again potentially confounded by previous antipsychotic treatment [28]. Thus, the initial [^{11}C]NSMP PET finding of greatly elevated striatal D_2 -receptor availability subsequently regressed to the mean in the compilation of many results, leaving a real effect, albeit of small magnitude. Interestingly, a very recent meta-analysis showed elevated variability of striatal $\text{D}_{2/3}$ receptor results (and likewise for DAT in the presynaptic terminals) of patients compared to healthy controls [47]. This suggests the presence of heterogeneous baseline dopamine $\text{D}_{2/3}$ receptor changes in patients, despite their shared diagnosis of schizophrenia. Such a phenomenon calls for studies of patient sample size sufficient to accommodate the biological variance and to support factor analysis, which might parse out disease subtypes in relation to specific symptom clusters, while correcting for effects of previous treatment with antipsychotic medication.

Unstated in the above account is the effect of competition from endogenous dopamine on the availability of dopamine $\text{D}_{2/3}$ sites in living brain. This is a well-established phenomenon for benzamide antagonist ligands and for certain agonists e.g. [56], but not for butyrophenones [57], such that amphetamine-evoked dopamine release increases the competition against the binding of many commonly used PET/SPECT ligands. Amphetamine challenge releases intraneuronal dopamine through a mechanism of facilitated exchange/diffusion. After

administration of a low dose of amphetamine, binding of the benzamide SPECT ligand [^{123}I]IBZM rapidly declined by about 20 % in unmediated schizophrenia patients, whereas the same treatment evoked only a 7 % decline in healthy controls [58], a finding which was replicated in a second cohort, albeit with slightly less group difference [59]. The greater variance of delta-BP_{ND} results in patients is again indicative of disease heterogeneity, and indeed, other research has shown a dependence of the amphetamine effect on the phase of illness, with greater dopamine release occurring in a state of worsening symptoms than during remission [38]. Partialling out the effect of symptom severity might help to discern the inherent magnitude of this relationship within a population.

Psychosocial stress evoked an elevated striatal dopamine release as measured by PET examination with the D₃-preferring agonist [^{11}C]PHNO in a group of (n = 25) immigrants with high risk for schizophrenia, as compared to a native-born control group [60]. In fact, the stress evoked a 3 % reduction in D_{2/3} availability in the control group, versus a 3% increase in the at-risk group, as compared to 15 % reduction in patients with schizophrenia. This stands somewhat in contrast to an earlier [^{11}C]raclopride PET study in schizotypal individuals [61], who are at high risk for developing psychosis. Here, the psychosocial stress evoked an apparent dopamine release only in the subgroup of schizotypal individuals with anhedonia, i.e. negative as distinct from positive schizotypy. This discrepancy might reflect the greater sensitivity of [^{11}C]PHNO binding (an agonist) to competition from endogenous dopamine, or might have more relation to neurochemical heterogeneity within the populations.

Hyper-responsivity of dopamine release to amphetamine challenge would most likely be a property of the presynaptic dopamine terminals. Behavioral sensitization to effects of amphetamine is a well-established phenomenon in experimental animals, and certainly has a presynaptic component. Furthermore, longitudinal [^{11}C]raclopride PET studies in healthy volunteers show a potentiation of the binding change after only a few repeated exposures to amphetamine as much as a year previously [62]. We have recently shown that mildly sensitizing amphetamine treatment potentiates the binding reduction of the D₃-preferring agonist ligand [^{11}C]PHNO binding evoked by amphetamine challenge in healthy controls, attaining a magnitude similar to that seen in un-sensitized patients with schizophrenia [63]. The parsimonious interpretation here is that repeated exposure to amphetamine induces a plastic change in the presynaptic terminals of healthy volunteers, thus enhancing the magnitude of the dopamine release evoked by a subsequent challenge. In this scenario, patients with schizophrenia are in a natural or pre-existing state of sensitization.

However, sensitization to effects of psychostimulants might also have a post-synaptic component, if the dopamine receptors are more abundant or show enhanced coupling to their signal transduction pathways. This latter scenario might occur if the post-synaptic receptors were in a high affinity state for binding dopamine and other agonists. Testing this hypothesis in a PET study using the D₃-preferring agonist ligand [^{11}C]PHNO failed to show any difference in binding between patient and healthy control groups [64]. Furthermore, there was no difference in the amphetamine-evoked reduction in the striatal binding of the D_{2/3} agonist ligand [^{11}C]NPA in groups of (n = 14) unmedicated patients and healthy controls, nor did the individual binding reductions in the patient group correlate with worsening of positive symptoms [65].

Increasing the interstitial dopamine levels through psychostimulant drug treatment or by performing certain cognitive tasking imparts greater competition against the binding of [^{11}C]raclopride and certain other tracers relative to the tonic dopamine competition prevailing at a baseline condition. The converse pharmacological challenge, to cause depletion of dopamine levels, can unmask this basal occupancy. While reserpine serves admirably for dopamine depletion in rodent studies [56], it can produce an excessive dopamine depletion, exceeding the safety margin for research studies in human volunteers. However,

partial inhibition of dopamine synthesis with α -methyl-*para*-tyrosine (AMPT) is a well-tolerated pharmacological approach for transiently inducing dopamine depletion in human volunteers. The striatal binding of [^{123}I]IBZM at striatal D_{2/3} receptors thus increases after AMPT treatment in proportion to the basal occupancy by dopamine. The magnitude of this increase was higher in a group of patients with schizophrenia than in healthy controls, thus revealing their elevated basal occupancy by dopamine [66]. However, the binding change was highly variable between patients, and showed a strong inverse relationship with the extent of dysphoria experienced in individuals due to the dopamine depletion [67]. Thus, low basal occupancy is a predictor for greater dysphoria experienced upon dopamine depletion, which may be relevant to the generally poor compliance of dysphoric patients treated with antipsychotic medication. Furthermore, anti-dopaminergic compounds may have less therapeutic effect in those experiencing dysphoria simply because they already have relatively low dopamine signaling. Basal occupancy findings are highly variable, both in healthy controls and in patients; meta-analysis of results in controls showed occupancy in the range of 8–21 %, with a weighted average of 11 % [68]. While this may reflect biological variability or the range of responses in a stressful environment, there are also methodological considerations at play. In particular, the individual pharmacodynamic response to oral AMPT treatment will depend on time of fasting and the total plasma concentration of large neutral amino acids at the time of molecular imaging.

Dynamic changes in receptor availability are not just a matter of competition from endogenous dopamine, but also involve agonist-induced internalization of receptors, such that they become inaccessible to benzamide ligands. The agonist can be dopamine itself, or agonist drugs such as quinpirole. In a [^{11}C]raclopride PET study in non-human primates, escalating doses of the agonist quinpirole promoted the internalization of D_{2/3} receptors, as shown by declining striatal BP_{ND}. Meanwhile, concomitant fMRI recordings showed a steady decline of the effect of quinpirole on neurovascular coupling, consistent with loss of externalized and functional receptors [69]. In a human study with repeated PET scanning, a single challenge with amphetamine reduced striatal [^{11}C]raclopride binding relative to baseline by 12 % at three hours, 10 % at six hours and 6 % at nine hours in healthy controls [70]. Thus, the receptor availability changes outlasted the psychostimulant effects of the drug, no doubt reflecting a receptor internalization process. Patients with schizophrenia in that study showed a trend towards potentiation and prolongation of the internalization effect, which might indicate a defect in receptor cycling. However, the variance in the binding changes was considerably higher in the patients, again raising the possibility of neurochemical heterogeneity within a single diagnostic entity. Indeed, there emerges from the molecular imaging literature a consistent picture of heterogeneity of schizophrenia with respect to the presence or absence of functional hyperdopaminergia. This issue has great bearing on the approximately 30 % of patients with schizophrenia patients who have poor therapeutic response to antipsychotic medication [71].

Dopamine D₁ receptors are nearly as abundant in striatum as are D_{2/3} receptors [72], and greatly predominate over D₂-like receptors in cerebral cortex, where they are linked to cognitive function. However, there is scant documentation of the status of D₁ sites in schizophrenia. One study with the partially selective D₁ antagonist ligand [^{11}C]SCH23390 showed no difference in striatal binding, but 13 % lower binding in dorsolateral prefrontal cortex (DLPFC) of a group of 18 never-medicated patients compared with 17 controls [73]. The authors' summary of the seven published D₁ PET studies showed that one investigation reported 20 % lower [^{11}C]SCH23390 binding in striatum of patients with schizophrenia compared to their unaffected twins, and similar reductions in widespread cortical regions [74]. However, these differences seemed to bear some relationship with the medication history of the patients.

A study comparing two D₁ ligands ([^{11}C]SCH23390 and [^{11}C]

NNC112) in the same patient and control groups showed 33 % lower D_1 availability in frontal, anterior cingulate and temporal cortex of the patients, and 20 % lower binding in striatum [75]. A similar study of the two ligands showed globally 5–10 % higher BP_{ND} in a small group of drug free or naïve patients with schizophrenia [76], thus in contrast to preclinical findings that the two ligands differed with respect to their behavior *in vivo* [77]. Other research comparing drug naïve and previously treated patients with schizophrenia showed 33 % higher [^{11}C] NNC112 BP_{ND} in DLPFC specifically in the drug-naïve patient group [78]. The higher D_1 -receptor availability in DLPFC of drug-free and -naïve patients correlated with poor performance of the n-back test of working memory [79], whereas there was no such relationship in healthy volunteers [80]. Findings with hybrid PET/fMRI imaging showed that cortical dopamine signaling via D_1 -receptors supports intracortical cross talk that engages cognitive resources in the performance of working memory [81]. In relation to cognitive function, there is a distinction between sensitivity of dopamine D_1 -receptors and their absolute abundance [82]. Thus, D_1 -receptor findings in schizophrenia are especially difficult to interpret, as ordinary PET methods do not separate availability/abundance of the receptors from their sensitivity, both of which may be abnormal.

5. Assessment of antipsychotic target engagement

The application of molecular imaging that has had the largest impact on clinical practice in psychiatry is the determination of occupancy of D_2 -like dopamine receptors by antipsychotic drugs. Although the pharmaceutical industry has been trying to develop a non-dopaminergic antipsychotic compound for decades, all clinically used drugs for the treatment of schizophrenia are still either antagonists or partial agonists at $D_2/3$ receptors. The assessment of $D_2/3$ receptor occupancy allows the determination of the clinically useful drug dose, and it is now a routine tool for drug development [83]. Molecular imaging helps in establishing the relationship between plasma levels of the respective drug and the proportion of target molecules occupied in brain over time [84]. Monitoring of clinical effects and adverse reactions as a function of occupancy provides important information about relationships between brain and plasma drug levels and dosage regimens, but gives also insights into mechanisms of drug action.

PET/SPECT occupancy studies based on the principle that the experimental pharmaceutical displaces the radiotracer, which itself binds to the target molecule at tracer concentrations, thus avoiding mass effects. The extent of this displacement relates to the baseline binding of the radiotracer in unblocked state. Consequently, the radioactivity in the

target region in the blocked vs. the unblocked state provides the target occupancy (in %) as follows [84]:

$$\text{Occupancy [\%]} = 100 - \left[\frac{\text{Tracer Binding}_{\text{blocked}}}{\text{Tracer Binding}_{\text{unblocked}}} \times 100 \right]$$

Because it is often not feasible to study patients with schizophrenia in medication-free state, the PET/SPECT examinations often reflect the pharmacologically blocked state only. Unblocked baseline data from healthy volunteers must then serve for calculating the occupancy, assuming that patients and controls differ only marginally with respect to $D_2/3$ receptor availability at baseline, an issue discussed in some detail above. Fig. 1 depicts occupancy at cerebral dopamine receptors as a function of the plasma concentrations of antipsychotic medications.

The earliest PET occupancy studies conducted in the late 1980s suggested that clinically effective doses of first-generation antipsychotics such as haloperidol occupy D_2 -like dopamine receptors in the human striatum in the range between 65 and 90 % [85]. A “therapeutic window” between 65 and 80 % striatal occupancy is now widely accepted for antipsychotic drug action, including most of the second-generation antipsychotics (SGAs) [86]. Results suggest a “ceiling” of about 65 % occupancy of striatal dopamine $D_2/3$ receptors for sufficient treatment response, although such a high occupancy does not necessarily mean that every patient sufficiently improves. Upon raising antipsychotic doses to provoke greater than 80 % occupancy at brain receptors, the risk for extrapyramidal side effects (EPS, an iatrogenic parkinsonian syndrome) increases substantially. There are certain exceptions to those general rules [87]. Thus, clozapine and quetiapine both bind with such low affinity to D_2 -like dopamine receptors that even at high plasma concentrations they practically never occupy striatal $D_2/3$ receptors to an extent that is associated with EPS [88].

Partial $D_2/3$ agonists, on the other hand, almost completely occupy $D_2/3$ receptors at clinically effective doses [89]. There is unequivocal evidence for nearly complete therapeutic occupancy for all three clinically available partial agonists, i.e. aripiprazole [90], brexpiprazole [91], and cariprazine [92]. Presumably, the full occupancy by partial agonists “clamps” dopaminergic signaling at some level sufficient to avoid EPS, while avoiding the excessive signaling perhaps occurring in patients with schizophrenia. In the converse condition, treatment of Parkinson’s disease with pramipexole (a full agonist) alleviates motor symptoms while obtaining only a very low occupancy in the basal ganglia [93].

The utility of occupancy studies in schizophrenia is by no means limited to applications for the dopamine system. Early on, researchers

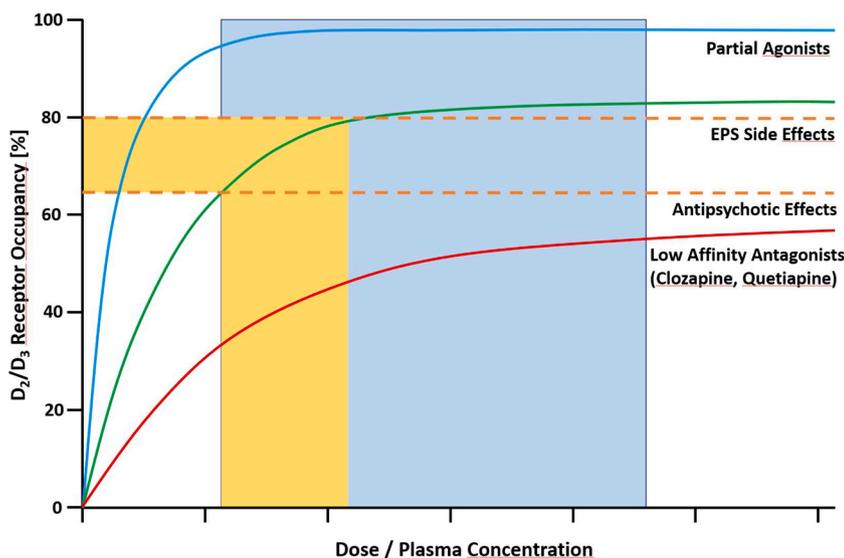


Fig. 1. Characteristic binding curves of antipsychotic drugs in human striatum as measured with PET. Dashed lines represent the 80 % threshold occupancy values for extrapyramidal symptoms (EPS) and the 65 % threshold for obtaining antipsychotic effects. The green line, showing that they fall within the 65–80 % “therapeutic window” of occupancy and therapeutic plasma concentrations, characterizes most antipsychotics, including most of the SGAs. The red line indicates occupancy by antipsychotics with low affinity for $D_2/3$ receptors. Even at high plasma concentrations, occupancies usually do not cross the 80 % threshold for extrapyramidal side effects (EPS). Such compounds exert antipsychotic effects despite their relatively low occupancy in the striatum. The blue binding curve is typical for all clinically available partial agonist antipsychotic medications; such medications have antipsychotic effects often at almost total saturation of $D_2/3$ receptors (in the flat part of the curve), as represented by the blue area. Presumably, complete occupancy by a partial agonist “clamps” the receptor activation at some level falling below the signaling by endogenous dopamine in the untreated condition.

exploited the ambivalent specificity of [^{11}C]NMSP (D_2 -like receptors in striatum versus serotonin $5\text{-HT}_{2\text{A}}$ receptors in cortex) to show that risperidone established roughly similar occupancy at both binding sites [94]. In recent elaboration of that approach, the occupancies by brexpiprazole at dopamine $\text{D}_{2/3}$ and serotonin $5\text{HT}_{1\text{A}}$ and $5\text{HT}_{2\text{A}}$ receptors and at serotonin transporters was investigated in a multitracer PET study [91]. SPECT studies with the muscarinic acetylcholine receptor ligand [^{123}I]-IDEX revealed 50 % occupancy by the SGA olanzapine, versus only 25 % occupancy by risperidone [95]. In general, the demonstration of off-target (non-dopaminergic) binding of an antipsychotic medication may be relevant to understanding its side-effect profile. Alternately, occupancy studies may establish engagement of a medication at new molecular targets, for example in an occupancy study of the potentially therapeutic drug tropisetron at $\alpha 7$ nicotinic receptors labelled with [^{11}C]CHIBA-1001 [96].

6. Brain serotonin markers

Post mortem neurochemical analysis in a small group of patients dying with schizophrenia suggested lower dopamine content and elevated serotonin metabolite levels in sporadic cases with paranoia, and higher levels of dopamine in a familial – formerly called hebephrenic – type schizophrenia [97]. While dopamine tissue content may bear little relationship with the dynamic aspect of dopamine measured by molecular imaging, the elevated serotonin finding might imply elevated turnover of that neurotransmitter. There have been no PET studies of serotonin synthesis in schizophrenia, although α -[^{11}C]methyl-*L*-tryptophan might serve for that purpose, at least in the dorsal raphe and thalamus, where the specific signal is highest [98].

The SPECT ligand [^{123}I] β -CIT binds ambivalently to DAT and serotonin transporters (SERT); due to their differing anatomic distributions, the signal in mesencephalon is attributable mainly to SERT, whereas the striatal signal mostly reflects DAT. A contrast of [^{123}I] β -CIT binding in 24 patients with schizophrenia and 22 healthy controls did not reveal any group difference in either transporter, despite the earlier demonstration of increased amphetamine-induced dopamine release in the same patients [99]. The PET ligand [^{11}C]DASB has largely supplanted the earlier SERT tracers, due to its selectivity and more sensitive depiction of the integrity of serotonin innervations. A [^{11}C]DASB study in ten schizophrenia patients and controls did not detect any group differences in SERT availability [100]. Another such study using high resolution PET to focus on SERT availability in the thalamus likewise revealed no group differences between schizophrenia patients contrasted with control subjects [101]. However, in the patient group there was a negative correlation between the severity of negative symptoms with SERT availability in the left anterior thalamic nucleus, which is a component of the Papez circuit for establishing declarative memory.

Another [^{11}C]DASB study showed no difference in SERT availability between a group of 19 presently unmedicated patients with schizophrenia contrasted with healthy controls [102]. FDG-PET in the same patients showed a typical pattern of cortical hypometabolism; interestingly, the control group manifested significant positive correlations between regional SERT in several high binding regions (thalamus, extended striatum) with FDG uptake in the same subcortical regions and a few cortical regions, and likewise between SERT in some cortical regions with cortical FDG uptake. The corresponding correlation matrices for the patients were very sparse. Thus, some modulation of cerebral energy metabolism by serotonin was seemingly lost in that group of patients with schizophrenia, although the causality is hard to establish.

Serotonin and dopamine innervations exhibit considerable overlap or convergence in the brain, notably in the basal ganglia, which raises the possibility of serotonergic modulation of dopamine release. Indeed, treatment with the mixed $5\text{HT}_{1\text{A}}/2\text{A}$ agonist psilocybin (a psychedelic substance) provoked amphetamine-like 20 % displacement of striatal [^{11}C]raclopride binding in a group of seven healthy volunteers [103]. The extent of depersonalization experienced by these individuals

correlated with the magnitude of their dopamine release, suggesting a mechanism whereby serotonin/dopamine interactions could contribute to psychotic symptoms. This background motivated a range of PET studies of post-synaptic serotonin receptors in schizophrenia. At least seven types exist in the brain, among which the best known are the $5\text{HT}_{1\text{A}}$ and $5\text{HT}_{2\text{A}}$ receptors.

The first PET study of serotonin receptors in schizophrenia detected no differences in the cortical binding of the $5\text{HT}_{2\text{A}}$ antagonist ligand [^{18}F]setoperone in a group of 14 medication free or naïve patients compared with healthy controls [104]. There were similar negative results in a subsequent [^{18}F]setoperone study comparing groups of ten patients and controls [105], and another study with 13 patients, of whom ten were native to antipsychotic medication [106]. One [^{18}F]setoperone study in six never-medicated patients reported a 16 % reduction in the frontal cortex [107], and there is a report of lower [^{18}F]altanserin binding in cerebral cortex of patients at risk for conversion, and low binding in caudate in the subset who did go on to develop schizophrenia [108]. Another study using [^{18}F]altanserin showed no abnormality in the cortical $5\text{HT}_{2\text{A}}$ binding in a group of 15 first episode, unmedicated patients, but did report a small increase in the caudate compared to the control group [109]. Upon expanding their sample to 30 patients, that research group reported 15 % lower BP_{ND} in the frontal cortex, specifically among the males. Furthermore, this decrease correlated with more severe positive symptoms [110]. However, they did not replicate in the larger sample their report of increased caudate binding, suggesting an earlier false positive finding.

These generally negative PET findings stand in contrast to *post mortem* binding study reporting a 28 % elevation in the binding of [^3H]ketanserin in frontal cortex membranes from ($n = 29$) never medicated patients, but no such difference in material from ($n = 16$) previously medicated patients [111]. The authors went on to measure the displacement of [^3H]ketanserin by a concentration series with the serotonin agonist DOI; their analysis indicated that samples from the unmedicated patients had a higher proportion of their binding sites in an agonist-binding state, which was likewise confirmed by a GTP-shift binding assay. This result predicts an elevation of $5\text{HT}_{2\text{A}}$ agonist binding in patients with schizophrenia, which might be testable in studies using new PET agonist ligands [112]. Alternately, the observations in brain membranes could predict enhanced hemodynamic response to $5\text{HT}_{2\text{A}}$ agonists in patients with schizophrenia, even if the availability of cortical receptors were unchanged or reduced, this in analogy to the fMRI/ D_2 receptor study with quinpirole challenge, cited above.

The $5\text{HT}_{1\text{A}}$ binding site is a somatodendritic autoreceptor expressed on serotonin neurons, but which is also abundantly present as a hetero-receptor in hippocampus. Several ligands are available for PET investigations of $5\text{HT}_{1\text{A}}$ sites, mainly finding use in studies of atypical antipsychotic medication occupancy, i.e. [113,114]. However, a [^{11}C]WAY 100,635 PET investigation of 22 medication-free patients with schizophrenia or schizoaffective disorder did not show any evidence of any difference in $5\text{HT}_{1\text{A}}$ binding compared to that in 18 healthy volunteers, despite *post mortem* results indicating elevated binding in pre-frontal cortex [115]. This negative results was preceded by studies showing 10–20 % higher [^{11}C]WAY 100,635 binding in temporal cortex of a group of 14 unmedicated patients [116], whereas another study with this ligand showed a small increase confined to the amygdala of the mostly drug-native group of 11 patients [117].

7. Acetylcholine receptors

There is sparse documentation of binding sites other than dopamine or serotonin receptors and transporters in the molecular imaging literature on schizophrenia. The nicotinic acetylcholine receptors (nAChR) have naturally drawn some attention, due to the self-medication hypothesis arising from the high incidence of smoking in clinical populations, although recent research questions the impact of smoking on cognition in schizophrenia [118]. A SPECT study investigated the

binding of the $\alpha 4\beta 2$ nAChR ligand [123 I]5-IA-85,380 in smokers with confirmed abstinence for one week, which is long enough to clear nicotine and its main metabolite. Comparison of ($n = 11$) patients with schizophrenia with a group of controls matched for tobacco consumption showed 25 % lower binding in cortex and thalamus of the withdrawn patients [119]. Since these binding sites are normally upregulated (and inactivated) by repeated nicotine exposure, that study suggested an abnormal responsiveness of the receptors among schizophrenia patients. A pilot study using 2- 18 F]FA, a ligand for the $\alpha 4\beta 2$ subtype of nAChRs showed 60 % reduced thalamic BP_{ND} for a group of ($n = 5$) patients with schizophrenia who were smokers, but this effect was attributed to simple competition from residual nicotine [120]. Another pilot PET study introducing the $\alpha 7$ -nAChR ligand [18 F]ASEM showed 10–20 % lower uptake (V_T , the total distribution volume; ml g⁻¹) in hippocampus of ($n = 6$) patients with schizophrenia (of which one smoker) compared with a larger control group [121].

Unlike the nicotinic receptors, which are ligand gated ion channels, the muscarinic acetylcholine receptors (mAChRs) are metabotropic G-protein receptors. PET or SPECT ligands are available for several subtypes among the five different mAChR families, but there have been relatively few molecular imaging investigations, other than occupancy studies focusing on atypical antipsychotic medications [95]. However, various observations link muscarinic transmission with certain schizophrenia symptoms [122], and *post mortem* analysis has shown a 25 % reduction in the M1 muscarinic binding site [123], and deficient allosteric modulation of N- 3 H]methylscopolamine binding *in vitro* [124]. Similarly, a SPECT study with [123 I]IQNB revealed 20 % lower M1 receptor binding in cortex and a 33 % reduction in caudate nucleus of a group of 12 medication free patients [125]. Another [123 I]IQNB study from the same group showed approximately 60 % receptor occupancy at M1 sites in patients treated with clozapine [126], but the cross sectional design could not distinguish simple occupancy from a down-regulation of the receptors due to treatment. A more recent [123 I]IDEX SPECT study in 30 medication-free patients showed a relationship between lower M1 muscarinic binding in dorsolateral prefrontal cortex with negative symptomatology and impairments in verbal memory [127].

8. Glutamate receptors

The glutamate receptors constitute a diverse family of ligand gated ion channels including the abundant NMDA receptors, and the metabotropic receptors, which are typical G-protein coupled receptors with seven transmembrane domains. There are relatively few molecular imaging studies of glutamate transmission in schizophrenia, despite the implicit importance of glutamatergic transmission in that disease. This is partially due to the historical lack of useful ligands for imaging the NMDA-type glutamate receptors, which has been something of a holy grail in radiopharmaceutical chemistry. Very recent preclinical reports introduced ligands for the NR2B subunit of the NMDA receptor [128], but the fitness of ligands proposed to target the open channel state of the NMDA receptor [129] is a matter of ongoing research [130]. Nonetheless, early SPECT studies with the NMDA receptor ligand [123 I]CNS-1261 were a high water mark in schizophrenia research of glutamatergic markers. In one of the first molecular imaging investigations of NMDA receptors in living human brain, [123 I]CNS-1261 revealed occupancy of NMDA sites by ketamine [131]. Administration of ketamine can acutely evoke some aspects of schizophrenia symptomatology in healthy individuals. Indeed, correlation analysis showed an association between the severities of negative symptom scores of the brief psychiatric rating scale with higher occupancy evoked by the same dose of ketamine in healthy volunteers [132]. In a SPECT study undertaken in schizophrenia patients who were under stable treatment with clozapine, the total distribution volume (V_T) of [123 I]CNS-1261 was globally reduced by one third relative to untreated patients and likewise healthy controls [133]. As noted above for the case of M1 muscarinic receptors, reduced NMDA availability could indicate simple competition of

clozapine at the binding sites, a down-regulation due to treatment, or a specifically disease-related decline in the binding site availability. Using a semiquantitative binding index for [123 I]CNS-1261, Pilowsky et al. found significantly reduced NMDA binding in the left hippocampus in drug-free patients with schizophrenia, and a less prominent reduction in those patients taking clozapine or typical antipsychotics at the time of scanning, whereas typical antipsychotic medication had no such effect on hippocampal binding [134]. Furthermore, her study showed a significant inverse correlation between [123 I]CNS-1261 binding and the severity of residual psychotic symptoms in those patients who were under treatment with typical antipsychotics. SPECT results in the medication-free subgroup of patients showed a significant positive correlation between [123 I]CNS-1261 binding in the middle inferior frontal cortex with the duration of illness duration. These results are generally in line with the NMDA receptor hypofunction hypothesis of schizophrenia and suggest that atypical antipsychotic treatment may contribute to alterations in NMDA receptor availability. These pioneering SPECT findings with [123 I]CNS-1261 call for renewed attention to the issue of NMDA receptor availability and their open channel state in the context of schizophrenia.

Glutamate also acts as a neurotransmitter at the eight members of the G-protein linked metabotropic glutamate receptor (mGluR) family, some of which may modulate other neurochemical factors implicated in schizophrenia. For example, the mGluR2 subtype forms a functional complex with cortical 5HT_{2A} receptors, and their activation interferes in the physiological and behavior effects of psychedelic drugs [135]. Pre-clinical findings implicate mGluR2 in the nucleus accumbens in the regulation of methamphetamine-induced dopamine release [136], which might be relevant to the dopamine sensitization state of schizophrenia. While subtype specificity has been hard to obtain, there are now promising tracers for mGluR1 [137], a lead compound for mGluR2 [138], and the successful tracer [11 C]ABP688 for mGluR5 imaging in human brain [139]. A study with this tracer showed 24 % lower [11 C]ABP688 binding in a group of ($n = 15$) patients with schizophrenia relative to a control group closely matched for age and smoking status [140]. This was despite the lack of compelling *post mortem* findings for mGluRs in schizophrenia, e.g. [141], perhaps reflecting allosteric modulation of the receptors in living brain, or alternately the poor test-retest reproducibility characteristic of [11 C]ABP688 PET.

9. Other neuroreceptors and biomarkers

Among the four known classes of receptors for opioid peptides, only the μ -subtype has been probed in PET studies with the selective agonist ligand [11 C]carfentanyl or less selective antagonist ligands such as [11 C]diprenorphine [142]. The only PET study of opioid receptors in schizophrenia showed 10 % reductions in [11 C]carfentanyl binding in striatum and the “hedonic network” in a group of ($n = 20$) patients, who also showed a markedly increased inter-regional correlation of binding compared with the control group [143]. The authors interpreted their results in light of the anhedonia and other negative symptoms of schizophrenia.

The tuberomammillary nucleus of the hypothalamus hosts a small population of histamine neurons, which innervate the forebrain. Of the four known types of histamine receptors, only the H₁ type (the site of action of sedating antihistamines) is yet amenable for study by PET, using the antagonist ligand [11 C]doxepin. The BP_{ND} of this ligand was 50 % lower in the prefrontal and cingulate cortices, but was unaffected in the thalamus, in a group of ($n = 10$) haloperidol-treated patients with schizophrenia compared with controls [144]. While atypical antipsychotic medications, especially those that cause weight gain, bind to H₁ sites, haloperidol is without great H₁ affinity. However, it could be that prolonged antipsychotic treatment down-regulates these binding sites by some mechanism.

There was recently a flurry of activity surrounding the hypothesis that neuroinflammation contributes to the pathology of schizophrenia.

Activated microglia and macrophages in brain express high levels of a marker known as TSPO in the mitochondrial membrane, which is detectable using a range of PET or SPECT tracers [145]. A meta-analysis of 12 TSPO PET studies using various tracers showed significantly higher signal in grey matter, which was of small effect size when calculated as BP_{ND} , but was not significant when calculated as V_T [146]. This discrepancy may be due to uncertainties in the validity of TSPO- BP_{ND} estimates, i.e. the lack of a true non-binding reference region. On the other hand, a follow-up study suggested that neuroinflammation to TSPO PET (quantified as V_T) may be a transient phenomenon in psychosis, perhaps related especially with the acute phase [147]. Other multimodal molecular imaging studies link microgliosis to TSPO PET in schizophrenia patients with attenuated extrastriatal dopamine release provoked by psychosocial stress [148], and to high levels of the inhibitory neurotransmitter GABA in frontal cortex, as measured by MR spectroscopy [149]. A further confounding factor in these studies is due to the bivalent nature of microglia, which can have pro-inflammatory or anti-inflammatory phenotypes. Application of tracers that target other specific aspects of neuroinflammation pathways may help resolve this issue [150].

Results of multimodal TSPO-PET/MR spectroscopy studies introduce the topic of molecular imaging of the nearly ubiquitous GABA-A binding sites with the benzodiazepine [^{11}C]flumazenil. Since GABA-A sites are so very abundant in the cerebral cortex, [^{11}C]flumazenil PET gives a surrogate index of neuronal density and cortical thickness in healthy brain [151]. Baseline [^{11}C] flumazenil V_T did not differ in groups of healthy controls and unmedicated patients with schizophrenia, which might indicate preservation of cortical architecture in schizophrenia [152]. However, group differences emerged upon pharmacological challenge with tiagabine, a blocker of the GABA plasma membrane transporter. This treatment normally elevates interstitial GABA levels, which increases the affinity of GABA-A sites for [^{11}C]flumazenil by an allosteric mechanism. Indeed, the tiagabine treatment provoked a 10–15 % increase in the [^{11}C]flumazenil V_T in the healthy control group, but no significant effect in the antipsychotic naïve patients. The authors proposed that this difference revealed an underlying defect in GABA-ergic signaling in schizophrenia. Another [^{11}C]flumazenil PET study showed 20 % lower V_T specifically in the right caudate of a group of individuals at extreme risk for developing psychosis [153], which had not been reported in the preceding study of patients with schizophrenia.

Post mortem investigation showed reduced protein levels of the metabotropic GABA-B receptors in patients with schizophrenia, but there were similar reductions in samples from other psychiatric populations [154]. There is a PET ligand for GABA-B sites validated for rodent studies [155], but at the time of writing, there are no molecular imaging studies of this target in schizophrenia patients.

Broadly speaking, the preponderance of neurotransmission is either inhibitory (GABA-ergic) or excitatory (glutamatergic), with additional modulation or shaping by biogenic monoamine and peptide neurotransmitters. A recent proposal integrates the original dopamine hypothesis of schizophrenia in a model positing disturbances in a triad of neurochemical networks: dopamine, serotonin, and glutamate [156]. In this scenario, abnormal glutamate-serotonin interactions in (frontal) cerebral cortex propagate to dysregulation of mesolimbic dopamine transmission. As noted above, patients, but not healthy controls, showed a very tight coupling between attenuated activation of BOLD signal in frontal cortex while performing a cognitive task, and elevated FDOPA uptake in the striatum [29]. Meta-analysis of many MR spectroscopy studies of glutamate/glutamine in schizophrenia indicates elevations in several brain regions [157]; we suppose that excessive excitatory transmission in frontal cortex may block the performance of certain cognitive tasks. A recent multimodal study investigated the relationship between cortical glutamate signals measured by MR spectroscopy with subcortical dopamine synthesis capacity to FDOPA PET in ($n = 28$) patients with schizophrenia [158]. In that study, the glutamate level in prefrontal cortex correlated inversely with FDOPA uptake in ventral

striatum, whereas there was a positive association with striatal glutamate levels. A completely independent multimodal imaging study of similar design substantially replicated the finding of an inverse association between cortical glutamate and subcortical dopamine [159], which furthermore showed a very strong correlation between positive symptom scores and cortical glutamate. Interestingly, others have reported that the stimulation of striatal perfusion to ^{15}O -water PET by treatment with antipsychotic medication correlated with baseline FDOPA utilization in striatum correlated with clinical improvement [160]. This could imply that a glutamate-mediated hyper-responsive perfusion increase in striatum predicts for a hyperactive dopamine system that could be amenable to treatment.

Ligands are now available for the molecular imaging of the synaptic vesicle protein 2A (SV2A), which provide an index of overall synaptic density. For example, [^{11}C]UCB-J sensitively detected a 40 % reduction in SV2A binding in patients with Alzheimer's disease [161]. In a group of ($n = 18$) patients with schizophrenia, [^{11}C]UCB-J PET revealed significantly lower binding of large effect size in frontal and anterior cingulate cortices, but no difference in hippocampus compared with the healthy control group [162]. The reductions were about 15 %, suggesting of widespread synaptic loss in those regions, although not to the same extent as in the frank neurodegeneration of Alzheimer's disease. We can predict that declines in cortical SV2A binding in schizophrenia should track the pattern of hypermetabolism seen in FDG PET.

Histone acetylase enzymes remove the acetyl group from histone proteins on DNA, thus interfering with the access of transcription factors and mediating epigenetic effects. A recent PET study with the non-selective histone acetylase ligand [^{11}C]martinostat indicated 50 % lower binding specifically confined to the dorsolateral prefrontal cortex of a mixed group of schizophrenia/schizoaffective patients [163]. There was a more widespread reduction in [^{11}C]martinostat binding in brain of patients with bipolar disorder, involving the thalamus, amygdala and hippocampus [164]. Thus, the focal finding in dorsolateral prefrontal cortex of schizophrenia might relate to working memory and executive function deficits of that condition. Here, the PET result of reduced histone acetylase binding overlaps with findings reported above for dopamine D_1 receptors and M1 muscarinic receptors.

10. Conclusion

Molecular imaging by PET or SPECT enables the non-invasive measurement of cerebral biomarkers across the spectrum extending from prodromal or UHR states preceding schizophrenia, to an acute phase, later remission from psychosis, and long-term outcome. In addition, test-retest molecular imaging studies allow dynamic assessments of responses to medications or psychosocial challenges. In contrast, *post mortem* examinations of brain usually reveal the end stage of a chronic illness, and bear the mark of perhaps decades of various treatment. Avoiding confounds due to treatment is logistically difficult in molecular imaging studies in schizophrenia, but may be the only way to separate trait, state, and medication effects. More than a generation of research in molecular imaging has failed to discover any marker that is pathognomonic of schizophrenia. While reduced blood flow and glucose metabolism in frontal brain regions are frequent findings, this kind of result is by no means diagnostic. Likewise, PET shows elevated capacity for dopamine synthesis in striatum in about half of cases, whereas $D_{2/3}$ receptor SPECT studies show elevated receptor occupancy at baseline and greater occupancy increases in response to amphetamine challenge, also in about half of cases. Presumably, the 50 % of schizophrenia patients with high dopamine synthesis are the same individuals who show greater responses to amphetamine challenge at post-synaptic receptors, and may be apt to have a better response (in positive symptoms) to antipsychotic medication. Furthermore, multimodal imaging studies suggest a link between elevated cortical glutamate and subcortical dopamine abnormalities. However, various lines of evidence indicate higher variance in dopaminergic measures among patients than in

control groups, which is itself suggestive of biochemical heterogeneity. When dopamine is not to blame, what is?

There are inconsistent findings of reduced serotonin 5HT_{2A} receptor availability in frontal cortex, which could hypothetically link impaired glutamatergic transmission in cortex and subcortical dopaminergic increases, as in the triad model recently proposed by Stahl [156]. Investigations of a wide range of other molecular imaging targets, usually in studies of small sample size, have shown either no differences, or small-moderate reductions in patients, i.e. cortical dopamine D₁, μ -opioid, histamine H₁, nicotinic, GABA-A, and mGluR5 receptors. In the absence of any particular hypothesis, PET researchers are seemingly seeking a highly sensitive neurochemical marker for schizophrenia by a process of elimination. In summary, four decades of molecular imaging studies of schizophrenia suggest a broad pathology affecting various neurotransmitter systems with overall loss of function, with the exception of a local over-activity of dopamine in striatum, and possibly in frontal cortex. Alternately, the brain of patients with schizophrenia may bear the marks of a decade of neurochemical aging in excess of chronological age, as shown by the small but significant declines reported for various other receptor types. Recent findings of reduced synaptic density reinforce this impression. Furthermore, inter-regional correlational analysis of PET results is widely used to tease out abnormal metabolic or neuroreceptor coupling, as distinct from absolute, statistically significant changes in receptor density, enzyme activity, or some other physiological parameter. What we call schizophrenia may remain a miasma of diffuse neurochemical changes more indicative of a generally ailing and functionally disconnected brain than of a singular disease pathology. However, this somewhat pessimistic view may yet yield to studies with new classes of receptor ligands.

A central aspect of the problem at hand is the unfitness of typical PET/SPECT studies in groups of perhaps 20 subjects to accommodate the high variability of endpoints, which may be due to disease heterogeneity, staging, and carryover of previous treatment effects. Although meta-analyses have been very successful in establishing the extent of changes in dopaminergic markers, we suppose that prospective multicenter studies may be required to obtain sufficient statistical power to parse out neurochemical subtypes of schizophrenia. In addition, a multimodal imaging approach may help to depict better the complexity of schizophrenia, as suggested by Stahl [156]. For example, a study combining structural and functional MR imaging with dual molecular imaging of dopamine synthesis capacity and glutamate receptors might support conclusions about heterogeneity of neurochemical and functional aspects of schizophrenia. Here, machine-learning techniques may reveal hidden aspects of the data, as demonstrated for the case of FDOPA [45]. This data-driven approach may help to simplify and resolve the hard trade-off between physiological resolution versus the logistics and costs of complex studies in large patient populations.

References

- [1] R.W. McCarley, et al., MRI anatomy of schizophrenia, *Biol. Psychiatry* 45 (9) (1999) 1099–1119.
- [2] M.E. Shenton, et al., A review of MRI findings in schizophrenia, *Schizophr. Res.* 49 (1–2) (2001) 1–52.
- [3] J.M.N. Duarte, L. Xin, Magnetic resonance spectroscopy in schizophrenia: evidence for glutamatergic dysfunction and impaired energy metabolism, *Neurochem. Res.* 44 (1) (2019) 102–116.
- [4] Pink spot in schizophrenia, *Br. Med. J.* 1 (5480) (1966) 119.
- [5] A.A. Boulton, C.A. Felton, The "pink spot" and schizophrenia, *Nature* 211 (5056) (1966) 1404–1405.
- [6] J.R. Stabenau, C.R. Creveling, J. Daly, The "pink spot," 3,4-dimethoxyphenylethylamine, common tea, and schizophrenia, *Am. J. Psychiatry* 127 (5) (1970) 611–616.
- [7] W. Choe, et al., Is this Red Spot the Blue Spot (locus ceruleus)? *Nucl. Med. Mol. Imaging* 44 (2) (2010) 157–158.
- [8] R.M. Cohen, et al., From syndrome to illness: delineating the pathophysiology of schizophrenia with PET, *Schizophr. Bull.* 14 (2) (1988) 169–176.
- [9] D.R. Weinberger, K.F. Berman, Speculation on the meaning of cerebral metabolic hypofrontality in schizophrenia, *Schizophr. Bull.* 14 (2) (1988) 157–168.
- [10] K. Hill, et al., Hypofrontality in schizophrenia: a meta-analysis of functional imaging studies, *Acta Psychiatr. Scand.* 110 (4) (2004) 243–256.
- [11] C.M. Clark, et al., Correlational methods for determining regional coupling of cerebral glucose metabolism: a pilot study, *Biol. Psychiatry* 19 (5) (1984) 663–678.
- [12] M. Katz, et al., Correlational patterns of cerebral glucose metabolism in never-medicated schizophrenics, *Neuropsychobiology* 33 (1) (1996) 1–11.
- [13] B.V. Siegel Jr., et al., Cortical-striatal-thalamic circuits and brain glucose metabolic activity in 70 unmedicated male schizophrenic patients, *Am. J. Psychiatry* 150 (9) (1993) 1325–1336.
- [14] S.G. Potkin, et al., A PET study of the pathophysiology of negative symptoms in schizophrenia. Positron emission tomography, *Am. J. Psychiatry* 159 (2) (2002) 227–237.
- [15] P. Flor-Henry, Psychosis and temporal lobe epilepsy. A controlled investigation, *Epilepsia* 10 (3) (1969) 363–395.
- [16] G.P. Reynolds, Increased concentrations and lateral asymmetry of amygdala dopamine in schizophrenia, *Nature* 305 (5934) (1983) 527–529.
- [17] D. Schuepbach, et al., Gender and rapid alterations of hemispheric dominance during planning, *Neuropsychobiology* 66 (3) (2012) 149–157.
- [18] A.J. Fallgatter, W.K. Strik, Reduced frontal functional asymmetry in schizophrenia during a cued continuous performance test assessed with near-infrared spectroscopy, *Schizophr. Bull.* 26 (4) (2000) 913–919.
- [19] J. Schroeder, et al., Patterns of cortical activity in schizophrenia, *Psychol. Med.* 24 (4) (1994) 947–955.
- [20] E.A. Hazlett, et al., Abnormal glucose metabolism in the mediodorsal nucleus of the thalamus in schizophrenia, *Am. J. Psychiatry* 161 (2) (2004) 305–314.
- [21] A.S. Mitchell, S. Chakraborty, What does the mediodorsal thalamus do? *Front. Syst. Neurosci.* 7 (2013) 37.
- [22] D.B. Calne, et al., Positron emission tomography after MPTP: observations relating to the cause of Parkinson's disease, *Nature* 317 (6034) (1985) 246–248.
- [23] B.E. Boyes, et al., Determination of plasma [18F]-6-fluorodopa during positron emission tomography: elimination and metabolism in carbidopa treated subjects, *Life Sci.* 39 (23) (1986) 2243–2252.
- [24] W.R. Martin, et al., Nigrostriatal function in humans studied with positron emission tomography, *Ann. Neurol.* 26 (4) (1989) 535–542.
- [25] P. Cumming, et al., The metabolism of [18F]6-fluoro-L-3,4-dihydroxyphenylalanine in the hooded rat, *J. Neurochem.* 48 (2) (1987) 601–608.
- [26] Y. Kumakura, P. Cumming, PET studies of cerebral levodopa metabolism: a review of clinical findings and modeling approaches, *Neuroscientist* 15 (6) (2009) 635–650.
- [27] J. Reith, et al., Elevated dopa decarboxylase activity in living brain of patients with psychosis, *Proc. Natl. Acad. Sci. U. S. A.* 91 (24) (1994) 11651–11654.
- [28] O.D. Howes, et al., The nature of dopamine dysfunction in schizophrenia and what this means for treatment, *Arch. Gen. Psychiatry* 69 (8) (2012) 776–786.
- [29] A. Meyer-Lindenberg, et al., Reduced prefrontal activity predicts exaggerated striatal dopaminergic function in schizophrenia, *Nat. Neurosci.* 5 (3) (2002) 267–271.
- [30] T. Kattagen, et al., Striatal dopamine and reward prediction error signaling in unmedicated schizophrenia patients, *Schizophr. Bull.* (2020).
- [31] Y. Kumakura, et al., Elevated [18F]fluorodopamine turnover in brain of patients with schizophrenia: an [18F]fluorodopa/positron emission tomography study, *J. Neurosci.* 27 (30) (2007) 8080–8087.
- [32] I. Vernaleken, et al., Modulation of [18F]fluorodopa (FDOPA) kinetics in the brain of healthy volunteers after acute haloperidol challenge, *Neuroimage* 30 (4) (2006) 1332–1339.
- [33] G. Grunder, et al., Subchronic haloperidol downregulates dopamine synthesis capacity in the brain of schizophrenic patients in vivo, *Neuropsychopharmacology* 28 (4) (2003) 787–794.
- [34] A.A. Grace, et al., Dopamine-cell depolarization block as a model for the therapeutic actions of antipsychotic drugs, *Trends Neurosci.* 20 (1) (1997) 31–37.
- [35] M. Avram, et al., Reduced striatal dopamine synthesis capacity in patients with schizophrenia during remission of positive symptoms, *Brain* 142 (6) (2019) 1813–1826.
- [36] G. Grunder, P. Cumming, The downside of downregulation, *Brain* 142 (6) (2019) 1500–1502.
- [37] S. Jauhar, et al., The effects of antipsychotic treatment on presynaptic dopamine synthesis capacity in first-episode psychosis: a positron emission tomography study, *Biol. Psychiatry* 85 (1) (2019) 79–87.
- [38] M. Laruelle, et al., Increased dopamine transmission in schizophrenia: relationship to illness phases, *Biol. Psychiatry* 46 (1) (1999) 56–72.
- [39] O.D. Howes, et al., Dopamine synthesis capacity before onset of psychosis: a prospective [18F]-DOPA PET imaging study, *Am. J. Psychiatry* 168 (12) (2011) 1311–1317.
- [40] A. Egerton, et al., Presynaptic striatal dopamine dysfunction in people at ultra-high risk for psychosis: findings in a second cohort, *Biol. Psychiatry* 74 (2) (2013) 106–112.
- [41] A. Egerton, et al., Adversity in childhood linked to elevated striatal dopamine function in adulthood, *Schizophr. Res.* 176 (2–3) (2016) 171–176.
- [42] J. Huttunen, et al., Striatal dopamine synthesis in first-degree relatives of patients with schizophrenia, *Biol. Psychiatry* 63 (1) (2008) 114–117.
- [43] A. Demjaha, et al., Dopamine synthesis capacity in patients with treatment-resistant schizophrenia, *Am. J. Psychiatry* 169 (11) (2012) 1203–1210.
- [44] E. Kim, et al., Presynaptic dopamine capacity in patients with treatment-resistant schizophrenia taking clozapine: an [(18F)FDOPA PET study, *Neuropsychopharmacology* 42 (4) (2017) 941–950.

- [45] M. Veronese, et al., A potential biomarker for treatment stratification in psychosis: evaluation of an 18 F DOPA PET imaging approach, *Neuropsychopharmacology* (2020).
- [46] E. Artiges, et al., Striatal and extrastriatal dopamine transporter availability in schizophrenia and its clinical correlates: a voxel-based and high-resolution PET study, *Schizophr. Bull.* 43 (5) (2017) 1134–1142.
- [47] S.P. Brugger, et al., Heterogeneity of striatal dopamine function in schizophrenia: meta-analysis of variance, *Biol. Psychiatry* 87 (3) (2020) 215–224.
- [48] D.J. Doudet, et al., Effect of age on markers for monoaminergic neurons of normal and MPTP-lesioned rhesus monkeys: a multi-tracer PET study, *Neuroimage* 30 (1) (2006) 26–35.
- [49] D. Amato, et al., A dopaminergic mechanism of antipsychotic drug efficacy, failure, and failure reversal: the role of the dopamine transporter, *Mol. Psychiatry* (2018).
- [50] D. Amato, et al., Hypofunctional dopamine uptake and antipsychotic treatment-resistant schizophrenia, *Front. Psychiatry* 10 (2019) 314.
- [51] D.F. Wong, et al., Positron emission tomography reveals elevated D2 dopamine receptors in drug-naïve schizophrenics, *Science* 234 (4783) (1986) 1558–1563.
- [52] A.L. Nordstrom, et al., No elevated D2 dopamine receptors in neuroleptic-naïve schizophrenic patients revealed by positron emission tomography and 11 C]-N-methylspiperone, *Psychiatry Res.* 61 (2) (1995) 67–83.
- [53] L. Farde, et al., D2 dopamine receptors in neuroleptic-naïve schizophrenic patients. A positron emission tomography study with 11 C]raclopride, *Arch. Gen. Psychiatry* 47 (3) (1990) 213–219.
- [54] J. Kambeitz, et al., Alterations in cortical and extrastriatal subcortical dopamine function in schizophrenia: systematic review and meta-analysis of imaging studies, *Br. J. Psychiatry* 204 (6) (2014) 420–429.
- [55] L.P. Kestler, E. Walker, E.M. Vega, Dopamine receptors in the brains of schizophrenia patients: a meta-analysis of the findings, *Behav. Pharmacol.* 12 (5) (2001) 355–371.
- [56] P. Cumming, et al., Specific binding of 11 C]raclopride and N- 3 H]propyl-norapomorphine to dopamine receptors in living mouse striatum: occupancy by endogenous dopamine and guanosine triphosphate-free G protein, *J. Cereb. Blood Flow Metab.* 22 (5) (2002) 596–604.
- [57] S.D. Ostergaard, et al., MDMA-evoked changes in the binding of dopamine D(2) receptor ligands in striatum of rats with unilateral serotonin depletion, *Synapse* 64 (1) (2010) 70–82.
- [58] M. Laruelle, et al., Single photon emission computerized tomography imaging of amphetamine-induced dopamine release in drug-free schizophrenic subjects, *Proc. Natl. Acad. Sci. U. S. A.* 93 (17) (1996) 9235–9240.
- [59] A. Abi-Dargham, et al., Increased striatal dopamine transmission in schizophrenia: confirmation in a second cohort, *Am. J. Psychiatry* 155 (6) (1998) 761–767.
- [60] A. Egerton, et al., Elevated striatal dopamine function in immigrants and their children: a risk mechanism for psychosis, *Schizophr. Bull.* 43 (2) (2017) 293–301.
- [61] A. Soliman, et al., Stress-induced dopamine release in humans at risk of psychosis: a 11 C]raclopride PET study, *Neuropsychopharmacology* 33 (8) (2008) 2033–2041.
- [62] I. Boileau, et al., Modeling sensitization to stimulants in humans: an 11 C]raclopride/positron emission tomography study in healthy men, *Arch. Gen. Psychiatry* 63 (12) (2006) 1386–1395.
- [63] A. Weidenauer, et al., On the relationship of first-episode psychosis to the amphetamine-sensitized state: a dopamine D2/3 receptor agonist radioligand study, *Transl. Psychiatry* 10 (1) (2020) 2.
- [64] A. Graff-Guerrero, et al., The dopamine D2 receptors in high-affinity state and D3 receptors in schizophrenia: a clinical 11 C]-(-)-PHNO PET study, *Neuropsychopharmacology* 34 (4) (2009) 1078–1086.
- [65] W.G. Frankle, et al., Amphetamine-induced striatal dopamine release measured with an agonist radiotracer in schizophrenia, *Biol. Psychiatry* 83 (8) (2018) 707–714.
- [66] A. Abi-Dargham, et al., Increased baseline occupancy of D2 receptors by dopamine in schizophrenia, *Proc. Natl. Acad. Sci. U. S. A.* 97 (14) (2000) 8104–8109.
- [67] L. Voruganti, et al., Subjective effects of AMPT-induced dopamine depletion in schizophrenia: correlation between dysphoric responses and striatal D(2) binding ratios on SPECT imaging, *Neuropsychopharmacology* 25 (5) (2001) 642–650.
- [68] F. Caravaggio, et al., What proportion of striatal D2 receptors are occupied by endogenous dopamine at baseline? A meta-analysis with implications for understanding antipsychotic occupancy, *Neuropharmacology* 163 (2020) 107591.
- [69] C.Y. Sander, et al., Imaging agonist-induced D2/D3 receptor desensitization and internalization in vivo with PET/fMRI, *Neuropsychopharmacology* 41 (5) (2016) 1427–1436.
- [70] J.J. Weinstein, et al., PET imaging of dopamine-D2 receptor internalization in schizophrenia, *Mol. Psychiatry* 23 (6) (2018) 1506–1511.
- [71] D.R. Gotfredsen, et al., Stability and development of psychotic symptoms and the use of antipsychotic medication - long-term follow-up, *Psychol. Med.* 47 (12) (2017) 2118–2129.
- [72] P. Cumming, Absolute abundances and affinity states of dopamine receptors in mammalian brain: a review, *Synapse* 65 (9) (2011) 892–909.
- [73] P. Stenkrona, et al., D1-dopamine receptor availability in first-episode neuroleptic naïve psychosis patients, *Int. J. Neuropsychopharmacol.* 22 (7) (2019) 415–425.
- [74] J. Hirvonen, et al., Brain dopamine D1 receptors in twins discordant for schizophrenia, *Am. J. Psychiatry* 163 (10) (2006) 1747–1753.
- [75] J. Kosaka, et al., Decreased binding of 11 C]NNC112 and 11 C]SCH23390 in patients with chronic schizophrenia, *Life Sci.* 86 (21–22) (2010) 814–818.
- [76] E.M. Poels, et al., In vivo binding of the dopamine-1 receptor PET tracers 11 C]NNC112 and 11 C]SCH23390: a comparison study in individuals with schizophrenia, *Psychopharmacology (Berl.)* 228 (1) (2013) 167–174.
- [77] N. Guo, et al., Dopamine depletion and in vivo binding of PET D1 receptor radioligands: implications for imaging studies in schizophrenia, *Neuropsychopharmacology* 28 (9) (2003) 1703–1711.
- [78] A. Abi-Dargham, et al., Increased prefrontal cortical D(1) receptors in drug naïve patients with schizophrenia: a PET study with 11 C]NNC112, *J. Psychopharmacol. (Oxford)* 26 (6) (2012) 794–805.
- [79] A. Abi-Dargham, et al., Prefrontal dopamine D1 receptors and working memory in schizophrenia, *J. Neurosci.* 22 (9) (2002) 3708–3719.
- [80] S. Karlsson, et al., Relationship of dopamine D1 receptor binding in striatal and extrastriatal regions to cognitive functioning in healthy humans, *Neuroimage* 57 (2) (2011) 346–351.
- [81] J.L. Roffman, et al., Dopamine D1 signaling organizes network dynamics underlying working memory, *Sci. Adv.* 2 (6) (2016) e1501672.
- [82] C. Wass, et al., Dopamine D1 sensitivity in the prefrontal cortex predicts general cognitive abilities and is modulated by working memory training, *Learn. Mem.* 20 (11) (2013) 617–627.
- [83] D.F. Wong, J. Tauscher, G. Grunder, The role of imaging in proof of concept for CNS drug discovery and development, *Neuropsychopharmacology* 34 (1) (2009) 187–203.
- [84] G. Grunder, et al., Therapeutic plasma concentrations of antidepressants and antipsychotics: lessons from PET imaging, *Pharmacopsychiatry* 44 (6) (2011) 236–248.
- [85] L. Farde, et al., Positron emission tomographic analysis of central D1 and D2 dopamine receptor occupancy in patients treated with classical neuroleptics and clozapine. Relation to extrapyramidal side effects, *Arch. Gen. Psychiatry* 49 (7) (1992) 538–544.
- [86] S. Nyberg, et al., Suggested minimal effective dose of risperidone based on PET-measured D2 and 5-HT2A receptor occupancy in schizophrenic patients, *Am. J. Psychiatry* 156 (6) (1999) 869–875.
- [87] G. Grunder, H. Hippus, A. Carlsson, The 'atypicality' of antipsychotics: a concept re-examined and re-defined, *Nat. Rev. Drug Discov.* 8 (3) (2009) 197–202.
- [88] G. Grunder, et al., The striatal and extrastriatal D2/D3 receptor-binding profile of clozapine in patients with schizophrenia, *Neuropsychopharmacology* 31 (5) (2006) 1027–1035.
- [89] G. Grunder, A. Carlsson, D.F. Wong, Mechanism of new antipsychotic medications: occupancy is not just antagonism, *Arch. Gen. Psychiatry* 60 (10) (2003) 974–977.
- [90] G. Grunder, et al., Brain and plasma pharmacokinetics of aripiprazole in patients with schizophrenia: an 18 F]fallypride PET study, *Am. J. Psychiatry* 165 (8) (2008) 988–995.
- [91] R.R. Girgis, et al., A positron emission tomography occupancy study of bexiprazole at dopamine D2 and D3 and serotonin 5-HT1A and 5-HT2A receptors, and serotonin reuptake transporters in subjects with schizophrenia, *Neuropsychopharmacology* 45 (5) (2020) 786–792.
- [92] R.R. Girgis, et al., Preferential binding to dopamine D3 over D2 receptors by cariprazine in patients with schizophrenia using PET with the D3/D2 receptor ligand 11 C]-(-)-PHNO, *Psychopharmacology (Berl.)* 233 (19–20) (2016) 3503–3512.
- [93] A. Deutschlander, et al., Occupancy of pramipexole (Sifrol) at cerebral dopamine D2/3 receptors in Parkinson's disease patients, *Neuroimage Clin.* 12 (2016) 41–46.
- [94] S. Nyberg, et al., 5-HT2 and D2 dopamine receptor occupancy in the living human brain. A PET study with risperidone, *Psychopharmacology (Berl.)* 110 (3) (1993) 265–272.
- [95] J. Lavalaye, et al., Higher occupancy of muscarinic receptors by olanzapine than risperidone in patients with schizophrenia. A 123 I]-IDEX SPECT study, *Psychopharmacology (Berl.)* 156 (1) (2001) 53–57.
- [96] M. Ishikawa, et al., Occupancy of alpha7 nicotinic acetylcholine receptors in the brain by tropisetron: a positron emission tomography study using 11 C]CHIBA-1001 in healthy human subjects, *Clin. Psychopharmacol. Neurosci.* 9 (3) (2011) 111–116.
- [97] L.O. Hansson, et al., Evidence for biochemical heterogeneity in schizophrenia: a multivariate study of monoaminergic indices in human post-mortal brain tissue, *J. Neural Transm. Gen. Sect.* 98 (3) (1994) 217–235.
- [98] P. Cumming, Measuring effects of MDMA (ecstasy) abuse on the rate of cerebral serotonin synthesis, *J. Neurochem.* 131 (5) (2014) 541–545.
- [99] M. Laruelle, et al., Dopamine and serotonin transporters in patients with schizophrenia: an imaging study with 123 I]beta-CIT, *Biol. Psychiatry* 47 (5) (2000) 371–379.
- [100] W.G. Frankle, et al., Serotonin transporter availability in patients with schizophrenia: a positron emission tomography imaging study with 11 C]DASB, *Biol. Psychiatry* 57 (12) (2005) 1510–1516.
- [101] J.H. Kim, et al., Serotonin transporter availability in thalamic subregions in schizophrenia: a study using 7.0-T MRI with 11 C]DASB high-resolution PET, *Psychiatry Res.* 231 (1) (2015) 50–57.
- [102] J.H. Kim, et al., Altered interregional correlations between serotonin transporter availability and cerebral glucose metabolism in schizophrenia: a high-resolution PET study using 11 C]DASB and 18 F]FDG, *Schizophr. Res.* 182 (2017) 55–65.
- [103] F.X. Vollenweider, et al., 5-HT modulation of dopamine release in basal ganglia in psilocybin-induced psychosis in man—a PET study with 11 C]raclopride, *Neuropsychopharmacology* 20 (5) (1999) 424–433.

- [104] C. Trichard, et al., No serotonin 5-HT_{2A} receptor density abnormality in the cortex of schizophrenic patients studied with PET, *Schizophr. Res.* 31 (1) (1998) 13–17.
- [105] R. Lewis, et al., Serotonin 5-HT₂ receptors in schizophrenia: a PET study using [¹⁸F]setoperone in neuroleptic-naïve patients and normal subjects, *Am. J. Psychiatry* 156 (1) (1999) 72–78.
- [106] N.P. Verhoeff, et al., A voxel-by-voxel analysis of [¹⁸F]setoperone PET data shows no substantial serotonin 5-HT_{2A} receptor changes in schizophrenia, *Psychiatry Res.* 99 (3) (2000) 123–135.
- [107] E.T. Ngan, et al., Decreased serotonin 2A receptor densities in neuroleptic-naïve patients with schizophrenia: A PET study using [(18)F]setoperone, *Am. J. Psychiatry* 157 (6) (2000) 1016–1018.
- [108] R. Hurlmann, et al., 5-HT_{2A} receptor density is decreased in the at-risk mental state, *Psychopharmacology (Berl.)* 195 (4) (2008) 579–590.
- [109] D. Erritzoe, et al., Cortical and subcortical 5-HT_{2A} receptor binding in neuroleptic-naïve first-episode schizophrenic patients, *Neuropsychopharmacology* 33 (10) (2008) 2435–2441.
- [110] H. Rasmussen, et al., Decreased frontal serotonin_{2A} receptor binding in antipsychotic-naïve patients with first-episode schizophrenia, *Arch. Gen. Psychiatry* 67 (1) (2010) 9–16.
- [111] C. Murguruza, et al., Dysregulated 5-HT_{2A} receptor binding in postmortem frontal cortex of schizophrenic subjects, *Eur. Neuropsychopharmacol.* 23 (8) (2013) 852–864.
- [112] I.N. Petersen, et al., Convergent (18)F-labeling and evaluation of N-benzyl-phenethylamines as 5-HT_{2A} receptor PET ligands, *Bioorg. Med. Chem.* 24 (21) (2016) 5353–5356.
- [113] J. Lerond, et al., Effects of aripiprazole, risperidone, and olanzapine on 5-HT_{1A} receptors in patients with schizophrenia, *J. Clin. Psychopharmacol.* 33 (1) (2013) 84–89.
- [114] N. Seneca, et al., Occupancy of dopamine D(2) and D(3) and serotonin 5-HT_{1A} receptors by the novel antipsychotic drug candidate, cariprazine (RGH-188), in monkey brain measured using positron emission tomography, *Psychopharmacology (Berl.)* 218 (3) (2011) 579–587.
- [115] W.G. Frankle, et al., Serotonin 1A receptor availability in patients with schizophrenia and schizo-affective disorder: a positron emission tomography imaging study with [¹¹C]WAY 100635, *Psychopharmacology (Berl.)* 189 (2) (2006) 155–164.
- [116] J. Tauscher, et al., Brain serotonin 5-HT_{1A} receptor binding in schizophrenia measured by positron emission tomography and [¹¹C]WAY-100635, *Arch. Gen. Psychiatry* 59 (6) (2002) 514–520.
- [117] F. Yasuno, et al., Decreased 5-HT_{1A} receptor binding in amygdala of schizophrenia, *Biol. Psychiatry* 55 (5) (2004) 439–444.
- [118] D.L. Boggs, et al., Minimal effects of prolonged smoking abstinence or resumption on cognitive performance challenge the “self-medication” hypothesis in schizophrenia, *Schizophr. Res.* 194 (2018) 62–69.
- [119] D.C. D’Souza, et al., Lower $\alpha 2^*$ -nicotinic acetylcholine receptor availability in smokers with schizophrenia, *Am. J. Psychiatry* 169 (3) (2012) 326–334.
- [120] J.R. Brasic, et al., Positron emission tomography experience with 2-[(1)(8)F]fluoro-3-(2(S)-azetidylmethoxy)pyridine (2-[(1)(8)F]FA) in the living human brain of smokers with paranoid schizophrenia, *Synapse* 66 (4) (2012) 352–368.
- [121] D.F. Wong, et al., Brain PET imaging of alpha7-nAChR with [¹⁸F]ASEM: reproducibility, occupancy, receptor density, and changes in schizophrenia, *Int. J. Neuropsychopharmacol.* 21 (7) (2018) 656–667.
- [122] S.P. Carruthers, C.T. Gurvich, S.L. Rossell, The muscarinic system, cognition and schizophrenia, *Neurosci. Biobehav. Rev.* 55 (2015) 393–402.
- [123] C. Vingerhoets, et al., The effect of the muscarinic M1 receptor antagonist biperiden on cognition in medication free subjects with psychosis, *Eur. Neuropsychopharmacol.* 27 (9) (2017) 854–864.
- [124] S. Hopper, et al., Widespread changes in positive allosteric modulation of the muscarinic M1 receptor in some participants with schizophrenia, *Int. J. Neuropsychopharmacol.* 22 (10) (2019) 640–650.
- [125] T.J. Raedler, et al., In vivo determination of muscarinic acetylcholine receptor availability in schizophrenia, *Am. J. Psychiatry* 160 (1) (2003) 118–127.
- [126] T.J. Raedler, et al., Central muscarinic acetylcholine receptor availability in patients treated with clozapine, *Neuropsychopharmacology* 28 (8) (2003) 1531–1537.
- [127] G. Bakker, et al., Relationship between muscarinic M1 receptor binding and cognition in medication-free subjects with psychosis, *Neuroimage Clin.* 18 (2018) 713–719.
- [128] L. Cai, et al., Evaluation of (11)C-NR2B-SMe and its enantiomers as PET radioligands for imaging the NR2B subunit within the NMDA receptor complex in rats, *J. Nucl. Med.* (2020).
- [129] C.J. McGinnity, et al., Initial evaluation of 18F-GE-179, a putative PET Tracer for activated N-methyl D-aspartate receptors, *J. Nucl. Med.* 55 (3) (2014) 423–430.
- [130] Y.H. Joo, et al., In vivo glucose metabolism and glutamate levels in mGluR5 knockout mice: a multimodal neuroimaging study using [(18)F]FDG microPET and MRS, *EJNMMI Res.* 10 (1) (2020) 116.
- [131] J.M. Stone, et al., Ketamine displaces the novel NMDA receptor SPET probe [(123)I]CNS-1261 in humans in vivo, *Nucl. Med. Biol.* 33 (2) (2006) 239–243.
- [132] J.M. Stone, et al., Relationship between ketamine-induced psychotic symptoms and NMDA receptor occupancy: a [(123)I]CNS-1261 SPET study, *Psychopharmacology (Berl.)* 197 (3) (2008) 401–408.
- [133] R.A. Bressan, et al., Impact of schizophrenia and chronic antipsychotic treatment on [¹²³I]CNS-1261 binding to N-methyl-D-aspartate receptors in vivo, *Biol. Psychiatry* 58 (1) (2005) 41–46.
- [134] L.S. Pilowsky, et al., First in vivo evidence of an NMDA receptor deficit in medication-free schizophrenic patients, *Mol. Psychiatry* 11 (2) (2006) 118–119.
- [135] J. Gonzalez-Maeso, et al., Identification of a serotonin/glutamate receptor complex implicated in psychosis, *Nature* 452 (7183) (2008) 93–97.
- [136] M. Haddar, et al., Regulatory system of mGluR group II in the nucleus accumbens for methamphetamine-induced dopamine increase by the medial prefrontal cortex, *Neuropsychopharmacol. Rep.* 39 (3) (2019) 209–216.
- [137] S. Li, Y. Huang, In vivo imaging of the metabotropic glutamate receptor 1 (mGluR1) with positron emission tomography: recent advance and perspective, *Curr. Med. Chem.* 21 (1) (2014) 113–123.
- [138] K. Kumata, et al., Synthesis and evaluation of 4-(2-fluoro-4-[(11)C]methoxyphenyl)-5-((2-methylpyridin-4-yl)methoxy)picolinamide for PET imaging of the metabotropic glutamate receptor 2 in the rat brain, *Bioorg. Med. Chem.* 27 (3) (2019) 483–491.
- [139] D. Elmenhorst, et al., In vivo and in vitro validation of reference tissue models for the mGluR(5) ligand [(11)C]ABP688, *J. Cereb. Blood Flow Metab.* 30 (8) (2010) 1538–1549.
- [140] F. Akkus, et al., Metabotropic glutamate receptor 5 neuroimaging in schizophrenia, *Schizophr. Res.* 183 (2017) 95–101.
- [141] N. Matosin, et al., Metabotropic glutamate receptor mGluR2/3 and mGluR5 binding in the anterior cingulate cortex in psychotic and nonpsychotic depression, bipolar disorder and schizophrenia: implications for novel mGluR-based therapeutics, *J. Psychiatry Neurosci.* 39 (6) (2014) 407–416.
- [142] P. Cumming, et al., A survey of molecular imaging of opioid receptors, *Molecules* 24 (22) (2019).
- [143] A.H. Ashok, et al., Reduced mu opioid receptor availability in schizophrenia revealed with [(11)C]-carfentanil positron emission tomographic imaging, *Nat. Commun.* 10 (1) (2019) 4493.
- [144] K. Iwabuchi, et al., Histamine H1 receptors in schizophrenic patients measured by positron emission tomography, *Eur. Neuropsychopharmacol.* 15 (2) (2005) 185–191.
- [145] P. Cumming, et al., Sifting through the surfeit of neuroinflammation tracers, *J. Cereb. Blood Flow Metab.* 38 (2) (2018) 204–224.
- [146] T.R. Marques, et al., Neuroinflammation in schizophrenia: meta-analysis of in vivo microglial imaging studies, *Psychol. Med.* 49 (13) (2019) 2186–2196.
- [147] L. De Picker, et al., State-associated changes in longitudinal [(18)F]-PBR111 TSPO PET imaging of psychosis patients: Evidence for the accelerated ageing hypothesis? *Brain Behav. Immun.* 77 (2019) 46–54.
- [148] C. Schifani, et al., Preliminary data indicating a connection between stress-induced prefrontal dopamine release and hippocampal TSPO expression in the psychosis spectrum, *Schizophr. Res.* 213 (2019) 80–86.
- [149] T. Da Silva, et al., GABA levels and TSPO expression in people at clinical high risk for psychosis and healthy volunteers: a PET-MRS study, *J. Psychiatry Neurosci.* 44 (2) (2019) 111–119.
- [150] V. Narayanaswami, et al., Emerging PET radiotracers and targets for imaging of neuroinflammation in neurodegenerative diseases: outlook beyond TSPO, *Mol. Imaging* 17 (2018), p. 1536012118792317.
- [151] C. la Fougere, et al., Where in-vivo imaging meets cytoarchitectonics: the relationship between cortical thickness and neuronal density measured with high-resolution [¹⁸F]flumazenil-PET, *Neuroimage* 56 (3) (2011) 951–960.
- [152] W.G. Frankle, et al., In vivo measurement of GABA transmission in healthy subjects and schizophrenia patients, *Am. J. Psychiatry* 172 (11) (2015) 1148–1159.
- [153] J.I. Kang, et al., Reduced binding potential of GABA-A/benzodiazepine receptors in individuals at ultra-high risk for psychosis: an [¹⁸F]-fluoroflumazenil positron emission tomography study, *Schizophr. Bull.* 40 (3) (2014) 548–557.
- [154] S.H. Fatemi, T.D. Folsom, P.D. Thuras, Deficits in GABA(B) receptor system in schizophrenia and mood disorders: a postmortem study, *Schizophr. Res.* 128 (1–3) (2011) 37–43.
- [155] R. Naik, et al., Synthesis and evaluation of a new (18)F-labeled radiotracer for studying the GABAB receptor in the mouse brain, *ACS Chem. Neurosci.* 9 (6) (2018) 1453–1461.
- [156] S.M. Stahl, Beyond the dopamine hypothesis of schizophrenia to three neural networks of psychosis: dopamine, serotonin, and glutamate, *CNS Spectr.* 23 (3) (2018) 187–191.
- [157] K. Merritt, et al., Nature of glutamate alterations in schizophrenia: a meta-analysis of proton magnetic resonance spectroscopy studies, *JAMA Psychiatry* 73 (7) (2016) 665–674.
- [158] T. Gleich, et al., Prefrontal and striatal glutamate differently relate to striatal dopamine: potential regulatory mechanisms of striatal presynaptic dopamine function? *J. Neurosci.* 35 (26) (2015) 9615–9621.
- [159] S. Jauhar, et al., The relationship between cortical glutamate and striatal dopamine in first-episode psychosis: a cross-sectional multimodal PET and magnetic resonance spectroscopy imaging study, *Lancet Psychiatry* 5 (10) (2018) 816–823.
- [160] D.P. Eisenberg, et al., Presynaptic dopamine synthesis capacity in schizophrenia and striatal blood flow change during antipsychotic treatment and medication-free conditions, *Neuropsychopharmacology* 42 (11) (2017) 2232–2241.
- [161] M.K. Chen, et al., Assessing synaptic density in Alzheimer disease with synaptic vesicle glycoprotein 2A positron emission tomographic imaging, *JAMA Neurol.* 75 (10) (2018) 1215–1224.

- [162] E.C. Onwordi, et al., Synaptic density marker SV2A is reduced in schizophrenia patients and unaffected by antipsychotics in rats, *Nat. Commun.* 11 (1) (2020) 246.
- [163] T.M. Gilbert, et al., PET neuroimaging reveals histone deacetylase dysregulation in schizophrenia, *J. Clin. Invest.* 129 (1) (2019) 364–372.
- [164] C.J. Tseng, et al., In vivo human brain expression of histone deacetylases in bipolar disorder, *Transl. Psychiatry* 10 (1) (2020) 224.