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Functional neuroimaging in schizophrenia: diagnosis and drug discovery

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Functional neuroimaging provides a direct way of investigating the pathophysiology of schizophrenia *in vivo*. The function of neurotransmitters implicated in schizophrenia, such as dopamine and glutamate, can be assessed using molecular imaging techniques such as positron emission tomography (PET), single-photon emission tomography (SPET) and magnetic resonance spectroscopy (MRS). Regional brain activity, particularly that associated with the cognitive processes and symptoms associated with the disorder, can be studied using functional magnetic resonance imaging (fMRI). Here, we focus on the potential for the use of these techniques in the diagnosis of schizophrenia and in the development of new drugs for its treatment.

Background

Schizophrenia is a severe psychiatric disorder that affects ~1% of the population and is one of the top ten causes of disability worldwide [1]. It usually begins in late adolescence or early adulthood and is characterised by positive psychotic symptoms, such as delusions and hallucinations and disorganised speech, and negative psychotic symptoms, such as emotional blunting and loss of drive. These are accompanied by cognitive impairments, particularly in memory and executive functions [2], and marked social and occupational dysfunction. The aetiology of schizophrenia is incompletely understood, but the disorder involves abnormal dopamine and glutamate transmission (Table 1, Figure 1), as well as structural and functional abnormalities in cerebral cortical and subcortical areas. However, a core pathophysiological abnormality that could account for all the different clinical features of the disorder has not been identified.

Schizophrenia is diagnosed on purely clinical grounds, yet none of its clinical features are pathognomonic [3]. The most widely used diagnostic criteria [4] require patients to have had certain combinations of symptoms continuously for at least six months. However, even when detailed criteria are used in conjunction with standardised diagnostic interviews, there is still significant variability between clinicians in diagnosing the disorder [5]. This could partly reflect the continued reliance on clinical but not neurobiological features and the possibility that the category itself does not correspond to a single disorder [6].

Treatment involves the use of antipsychotic drugs (Table 2), all of which act as antagonists at central dopamine D2 receptors [7], although some have additional effects at other receptors. Treatment is effective at reducing the severity of positive psychotic symptoms but has limited impact on negative symptoms, cognitive impairments or the course of the disorder. Furthermore, about one-third of patients are resistant to first-line antipsychotic drugs. Clozapine is the only medication licensed for treatment resistance and, although its efficacy is superior to other antipsychotics, its use entails haematological monitoring to avoid serious adverse effects. Poor tolerability is a problem with all antipsychotic drugs and is a major issue because patients often have to take treatment for many years. There is thus a clear need for the discovery of new drugs to treat schizophrenia.

The aim of this article is to discuss the potential utility of functional neuroimaging in the diagnosis of schizophrenia and the development of new drugs for its treatment.

Functional neuroimaging techniques used to study schizophrenia

Regional cerebral blood flow

Functional magnetic resonance imaging (fMRI) (Figure 2) and other imaging techniques that measure regional cerebral blood flow have demonstrated that resting neural activity and activation during a variety of cognitive tasks are abnormal in several brain areas in schizophrenia. These brain areas include the prefrontal, cingulate and temporal cortex, the hippocampus, the striatum, the thalamus and the cerebellum. These abnormalities are small in magnitude and are not evident in all patients within a given sample [6], making it difficult to use them as a diagnostic aid in an individual patient. A further complicating factor is that positive symptoms, disorganised speech and negative symptoms are present to varying degrees in different patients with schizophrenia and are associated with distinct patterns of regional cortical activity [8–10]. At one stage it was hoped that 'hypofrontality', a reduction in task-related activation in the prefrontal cortex, might be pathognomonic for schizophrenia.

Table 1. Neurotransmitter dysfunction in schizophrenia

Neuro-transmitter	Abnormality at molecular level[a]	Associated clinical features	Corresponding drug treatment
Dopamine	Increased dopamine synthesis and release; ?Altered density of dopaminereceptors	Positive psychotic symptoms	Antagonism of dopamine D2 receptors (applies to all drugs in Table 2)
Glutamate	?Increased glutamate activity; ?Reduced activity at NMDA receptor	Disorganised speech; Negative symptoms; Impaired cognitive function	Reduction of glutamate release

[a]'?' indicates contrasting findings.

However, subsequent research revealed that this was an inconsistent finding, varying with the cognitive task being studied, how well patients performed the task and with the symptom profile at the time of scanning [11].

An alternative approach to identifying functional abnormalities in particular brain regions has been to look for abnormalities in the integration of function between brain regions, such as the prefrontal and temporal cortex [12]. Although there appear to be abnormalities in functional connectivity in schizophrenia, a single pattern of dysconnectivity that characterises its pathophysiology has yet to be identified. Other neuroimaging approaches currently under evaluation are intended to find spatial [e.g. support vector machines (SVMs)] [13] or spatial-temporal (dynamic discrimination analiysis) [14] features of brain activity that can be used to discriminate patients from controls.

Molecular imaging
Molecular neuroimaging studies permit the examination of chemical changes in the brain related to schizophrenia. Positron emission tomography (PET) and single-photon emission tomography (SPET) employ radioactive tracers to generate images reflecting the distribution of ligands for specific molecules in the brain, and they can be used to study the synthesis and release of neurotransmitters and the availability of neurotransmitter receptors (Figure 2). Magnetic resonance spectroscopy (MRS) uses magnetic resonance imaging (MRI) to measure the concentration of specific molecules within the brain (Figure 2).

Molecular imaging studies in schizophrenia are consistent with the notion that dopamine dysregulation is a key pathophysiological feature of the disorder (Table 1). Thus PET and SPET studies indicate that schizophrenia is associated with increased pre-synaptic striatal dopamine synthesis and storage [15–18] and increased striatal release of dopamine following amphetamine administration [19,20] (Figure 1). Whether there is also an increase in the density of striatal D2 receptors is less clear [21–24], although meta-anlayses suggest that there is a modest increase [25,26]. These dopaminergic neuroimaging abnormalities are not found in depression [27,28], but the extent to which they are specific to schizophrenia, as opposed to other disorders that involve psychosis [29] or an increased vulnerability to psychosis [30], remains unclear.

Contemporary theories of schizophrenia implicate other neurotransmitters in addition to dopamine (Table 1). When healthy people take uncompetitive antagonists for the NMDA (*N*-methyl-D-aspartate) receptor (such as PCP (phencyclidine) or ketamine) they develop transient positive and negative psychotic symptoms and cognitive impairments that resemble those of schizophrenia [31,32]. NMDA receptor blockade on GABAergic interneurons can lead to a disinhibition of glutamatergic projection neurons and elevated glutamate release [33]. In rodents this can lead to glutamate-dependent neurodegeneration [34]. It is hypothesised that similar mechanisms could occur in schizophrenia, through dysfunction of NMDA receptors or the GABAergic interneurons upon which they are expressed (Figure 1). Elevated glutamate is of particular interest as a pathophysiological mechanism, as it could contribute to the reductions in grey matter volume that are seen in MRI studies of schizophrenia through modulation of plasticity or even excitotoxiticy [35].

Neuroimaging studies have provided support for this NMDA receptor hypofunction model. A SPET study has reported reduced NMDA receptor binding in the hippocampus in schizophrenia [36], and MRS studies have found elevated glutamine (a marker of glutamate release) in the medial frontal cortex in patients with first episode schizophrenia [37] and in adolescents at high risk of schizophrenia [38]. By contrast, reduced anterior cingulate glutamine has been described in chronic schizophrenia [39], suggesting that the nature of the glutamate dysfunction might differ between the early and later phases of the disorder. MRS and fMRI studies respectively indicate that administration of ketamine (an NMDA receptor antagonist that induces symptoms of schizophrenia) is associated with an increase in anterior cingulate glutamine levels [40] and increased activation of the prefrontal and anterior cingulate cortex [41,42].

Neuroimaging abnormalities that predate the onset of schizophrenia
Some neuroimaging abnormalities in schizophrenia are evident before the onset of the disorder [5]. People with prodromal symptoms who have a high risk of developing schizophrenia show abnormalities of prefrontal and anterior cingulate function [43,44] and increased striatal dopamine levels [45] that are qualitatively similar to the changes seen in established schizophrenia. These findings raise the possibility that neuroimaging could be used to detect pathophysiological changes associated with the disorder before the onset of frank illness. This is of particular clinical interest because only a proportion of people with prodromal symptoms go on to develop schizophrenia, and neuroimaging might facilitate the targeting of novel preventative treatments [46,47] to this subgroup. This is discussed further in the context of drug discovery (see 'Drug discovery' below).

Clinical utility of neuroimaging in schizophrenia
The ability of neuroimaging to detect pathophysiolgical changes in advance of clinical symptoms points to its potential as an aid to diagnosis. However, the absence of a core pathophysiological abnormality in schizophrenia
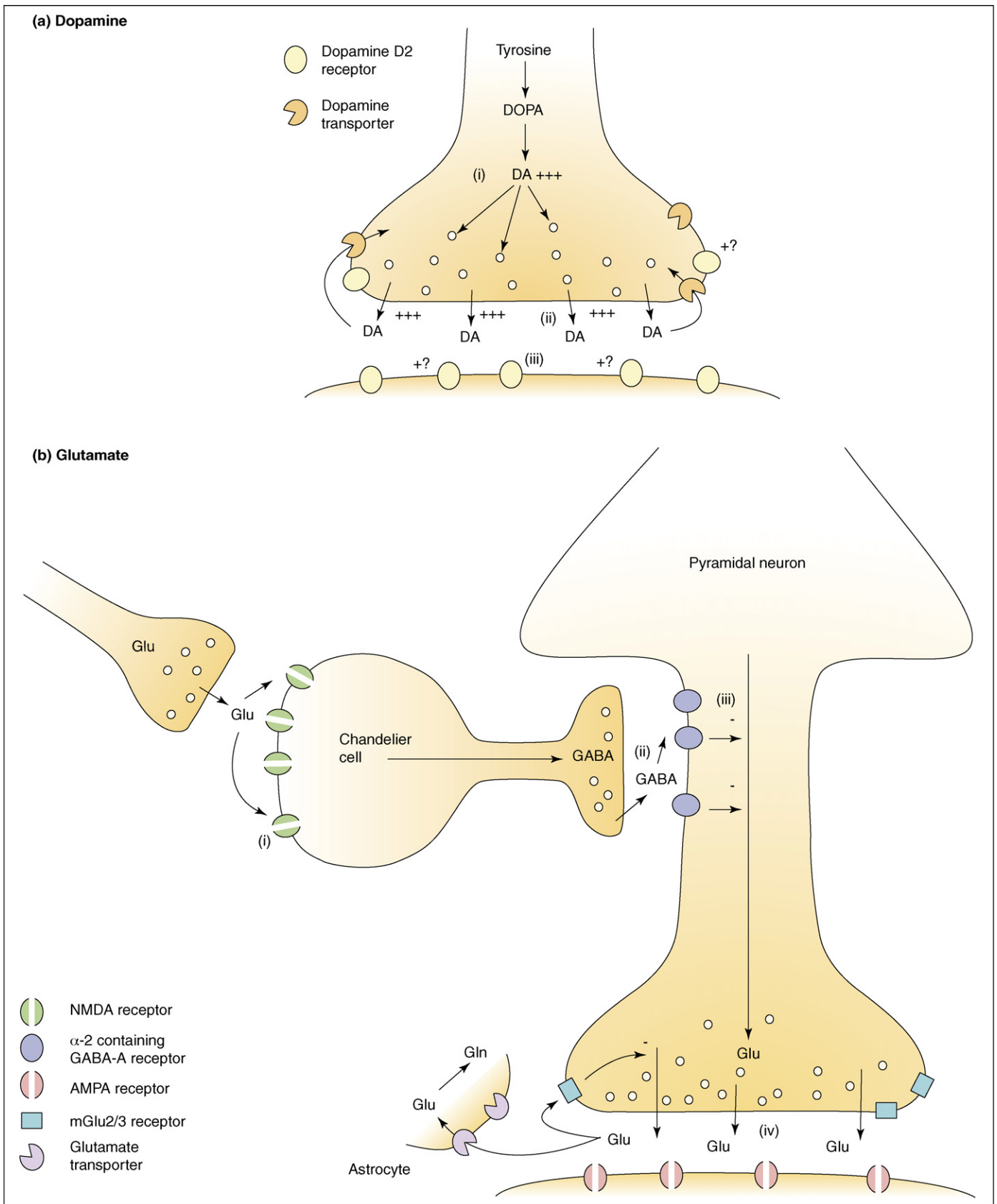
(a) Dopamine

(b) Glutamate

Figure 1. Abnormalities of neurotransmitter systems in schizophrenia. **(a)** Dopamine synthesis (i) and synaptic release (ii) are increased, whereas levels of D2 receptors are either normal or only modestly elevated (iii). **(b)** NMDA receptor hypofunction model. GABAergic interneurons that express NMDA receptors (i) are functionally impaired, leading to a reduction of GABA release onto pyramidal neurons (ii). The resultant disinhibition (iii) increases pyramidal neuron activity and the release of glutamate onto non-NMDA (AMPA) glutamate receptors (iv).

Table 2. Commonly used antipsychotic drugs

Typical (first generation) antipsychotics	Atypical (second generation) antipsychotics
Chlorpromazine	Clozapine
Haloperidol	Amisulpride
Trifluoperazine	Risperidone
Perphenazine	Olanzapine
Flupentixol	Quetiapine
Fluphenazine	Aripiprazole
Zuclopenthixol	

makes it difficult to use neuroimaging as a diagnostic tool. Neuroimaging abnormalities in schizophrenia are present to varying degrees in different patients, varying, for example, with variation in symptom profile. Moreover, some of the neuroimaging differences between patients and controls are quantitative rather than qualitative. The clinical applicability of functional neuroimaging in schizophrenia is also constrained by the limited availability and high cost of some forms of scanning, such as PET.

Drug discovery

Traditionally, the development of new drugs for the treatment of schizophrenia has relied on preclinical animal models. However, these models rely on our incomplete understanding of the pathophysiology of the disorder, and schizophrenia involves cognitive processes (such as language) and symptoms (such as auditory hallucinations and delusions) that do not occur in other species. Animal models have thus had limited success in predicting the efficacy of new compounds in patients [48].

Studies of existing antipsychotics

Neuroimaging has contributed substantially to our understanding of the mechanisms of action of existing antipsychotic drugs. SPET and PET studies have shown that, for many antipsychotics, therapeutic efficacy is associated with an occupancy of striatal D2 receptors of around 70%. Increasing the dose such that occupancy increases beyond this level increases the incidence of adverse effects but is not associated with additional efficacy [49]. These findings have helped to convey the notion to clinicians that
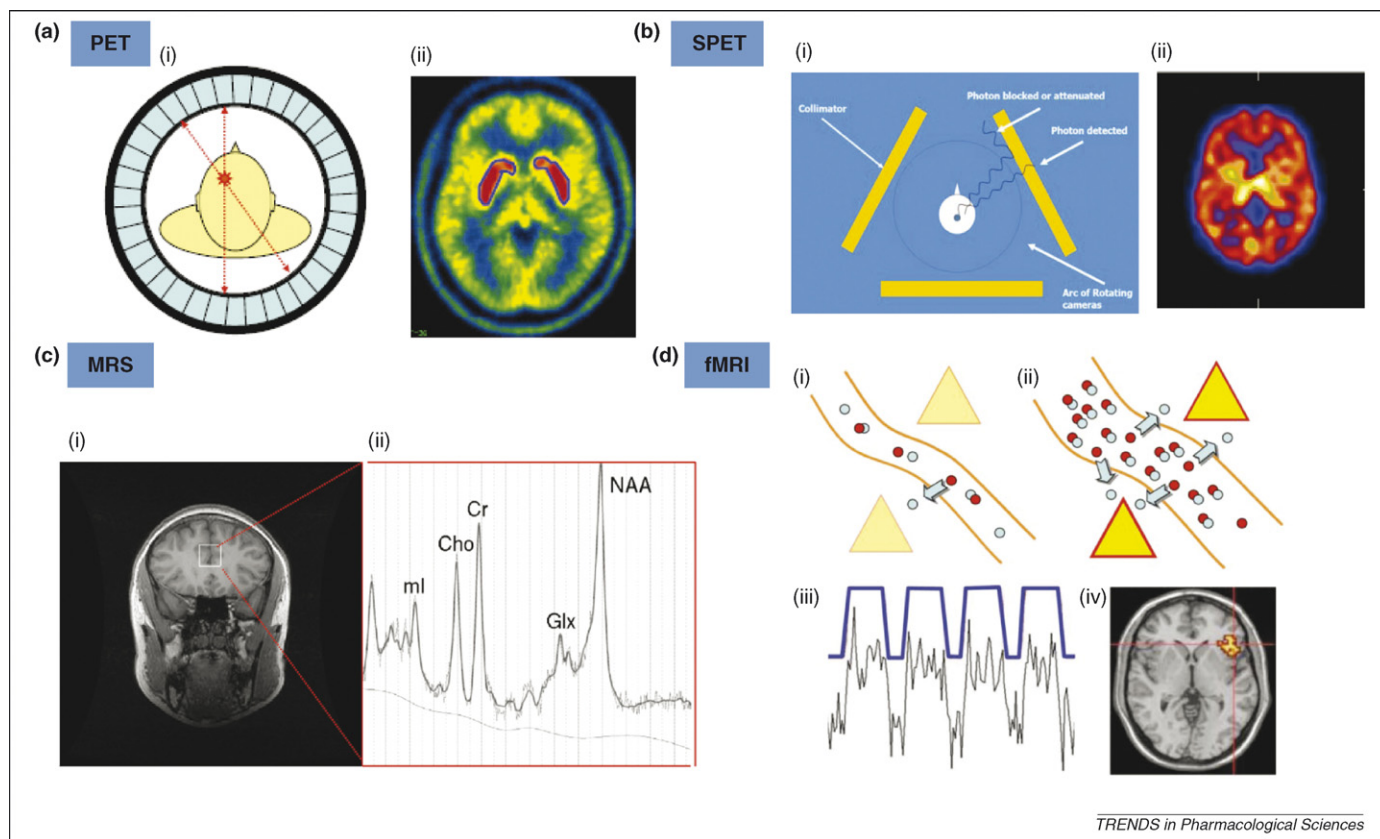

Figure 2. Basis of functional neuroimaging techniques. **(a)** Positron emission tomography (PET) and **(b)** Single-photon emission tomography (SPET) can be used to measure brain receptor availability, enzyme activity and drug–receptor interactions. Both techniques involve administration of a radiotracer, a molecule that has been labelled with radioactive isotope and binds to target of interest, such as a receptor. PET ligands emit positrons (a,i), which generate a pair of photons that travel in diametrically opposite directions, whereas SPET ligands emit a single photon (b,i). The scanners contain detectors positioned around the head that are sensitive to photons released from the radiotracer. Information from the detectors is used to construct an image that shows the distribution of the tracer in the brain (a,ii and b,ii). **(c)** Proton magnetic resonance spectroscopy (MRS) measures the relative concentration of different chemicals in the brain. When exposed to a magnetic field, chemicals respond differently according to the number and location of protons in their molecular structure. Within a region of interest in the brain (indicated by the box in (c,i)), MRS exploits this property to yield a spectrum of peaks (c,ii) corresponding to the different chemicals present. MRS can thus be used to estimate the local concentration of glutamate and glutamine (Glx), N-acetyl aspartate (NAA), creatine (Cr), myoinositol (mI) and choline (Cho). **(d)** Functional magnetic resonance imaging (fMRI) measures the BOLD (blood oxygenation level dependent) response. An increase in neural activity in response to an experimental stimulus increases local blood flow to provide more oxygen (blue circles), delivered as oxyheamoglobin (red and blue circles together). Blood flow during normal neural activity is shown in (d,i), whereas (d,ii) shows blood flow during increased neural activity. Oxy- and deoxyheamoglobin have different paramagnetic qualities, and the local change in their ratio results in signal that can be detected by the MRI scanner. As shown in (d,iii), the BOLD response (black line) varies in the presence and absence of the stimulus (blue line). (d,iv) depicts where in the brain there was a statistically significant change in activation, superimposed on a neuroanatomical image.

once this therapeutic threshold has been reached, further increases in dose are unlikely to improve the clinical response. Subsequent research has revealed that certain antipsychotic drugs, such as clozapine, are effective at lower levels of striatal D2 occupancy, suggesting that efficacy could also be related to effects at other receptors (see 'Novel antipsychotic drug targets' below). Lower striatal D2 occupancy might also account for the lower incidence of extrapyramidal side effects with clozapine compared to other antipsychotics.

Neuroimaging studies of the dopamine system have also provided data that indicate new ways that drugs could be used to treat schizophrenia. Presynaptic striatal dopamine availaibility and synaptic striatal dopamine release are elevated in schizophrenia [17,19], whereas striatal D2 levels are, at most, only modestly increased [25]. This suggests that medications that reduce pre-synaptic dopamine availability and release could be useful and that existing drug treatments, which target post-synaptic D2 receptors, could be acting downstream of the primary dopaminergic abnormality.

Although much research has focused on striatal D2 receptors, it has been suggested that the primary site of therapeutic action for antipsychotics might be D2 receptors in the cerebral cortex [50]. This is consistent with a meta-analysis of PET and SPET studies that found that the therapeutic dose of antipsychotics was more related to cortical than striatal D2 receptor occupancy [51]. SPET and PET studies have also reported that clozapine and antipsychotics with a low incidence of extrapyramidal side effects bind preferentially to temporal cortical over striatal D2 receptors [52], although this has not been a consistent finding [53,54]. Within the striatum, these antipsychotics have been found to bind preferentially to non-motor sub-regions [55], which might account for their reduced propensity to extrapyramidal side effects.

fMRI studies and studies of cerebral blood flow can be used to examine the influence of antipsychotics on regional brain activity and on activation during cognitive tasks and thus indicate their neurocognitive effects. In general, treatment with atypical compounds (Table 2) has been associated with greater regional cortical activity compared to treatment with typical antipsychotic drugs, whereas the latter have been associated with relatively more striatal activity [56,57]. This is consistent with evidence that atypical antipsychotics might have a greater effect on cognitive impairments in schizophrenia than typical drugs but are less likely to be associated with extrapyramidal side effects [58].

Novel antipsychotic drug targets
Clozapine is the most effective antipsychotic drug currently available [59]. Its actions at a wide variety of receptor subtypes in addition to dopamine D2 receptors, including antagonism at serotonin 5-HT$_{2A}$ receptors and agonism at dopamine D1 and muscarinic M1 and M4 receptors, suggest its efficacy might be related to binding at these sites [52]. Neuroimaging has been used to test this hypothesis. Initial studies investigated binding to 5-HT$_{2A}$ receptors but found that even at sub-therapeutic doses these were almost completely occupied by clozapine.

Clinical trials of pure 5-HT$_{2A}$ antagonists have also been disappointing [60]. SPET studies have reported that muscarinic receptor concentrations are markedly reduced in schizophrenia [61], consistent with post-mortem data [62], and that clozapine shows very high muscarinic receptor occupancy [63]. Furthermore, partial muscarinic M1 agonists appear to have an antipsychotic effect [64]. The M1 receptor might thus be a target for future antipsychotic drug development [52]. PET studies of D1 receptors suggest an increased density in schizophrenia [65], although this has not been a consistent finding [66]. D1 receptor agonists improve cognitive performance in experimental animals, suggesting a role in ameliorating cognitive impairments in schizophrenia. However, at present no drugs that primarily act at D1 receptors have been developed for clinical use.

Data from recent SPET and MRS studies of the glutamate system in schizophrenia (see 'Molecular imaging' above) are consistent with the NMDA receptor hypofunction hypothesis of schizophrenia (Table 1, Figure 1), suggesting that enhancement of NMDA receptor function could be a target for new antipsychotic compounds. However, drugs that have this effect through agonism at the receptor's glycine binding site lead to only a modest improvement in negative symptoms when used as add-on agents to conventional antipsychotic drugs [67]. An alternative approach is to develop drugs that correct the downstream effects of putative NMDA receptor dysfunction (Figure 1). Lamotrigine, an antiepileptic drug that reduces presynaptic glutamate release, was found, in a small double blind randomised placebo controlled study, to improve both psychotic and negative symptoms in schizophrenia [68]. Furthermore, a recent phase II trial of a pure metabotropic glutamate 2/3 (mGlu2/3) agonist (which would be predicted to reduce glutamate release) found that it had similar efficacy to olanzapine, an established antipsychotic [69]. At present, the mechanism of action of these putative antipsychotics is inferred from animal and *in vitro* studies. Neuroimaging studies of these compounds could be used to show how they act on the brain in humans, which would inform future drug development in this area.

Cannabis use can induce acute psychotic symptoms and regular use might increase the risk of developing schizophrenia [70]. Neuroimaging studies suggest that its induction of psychotic symptoms is related to effects of Δ^9-tetrahydrocannabinol (Δ^9-THC) on activity in the prefrontal and anterior cingulate cortex and the striatum [71]. These effects of Δ^9-THC are mediated by its binding to the CB1 receptor, suggesting that compounds that act this site could have antipsychotic efficacy. A trial of a CB1 receptor antagonist proved disappointing [72], but cannabidiol (CBD), a component of the cannabis plant that does not appear to act on CB1 receptors, has been reported to have an antipsychotic effect [73]. Neuroimaging studies indicate that CBD modulates function in the cingulate cortex and amygdala [74], although this was associated with anxiolytic rather than antipsychotic effects.

Neuroimaging and vulnerability to schizophrenia
The aetiology of schizophrenia has a major genetic component, and it is thought to involve multiple genes with

relatively small effects [75]. To date, the genes that have been most consistently linked with the disorder are *DISC1*, *dysbindin* and *neuregulin*. Neuroimaging can be used to examine how a gene normally influences brain function by comparing data from subjects categorised according to the presence or absence of the risk variant of that gene. Recent studies that have used this approach suggest that *DISC1* influences hippocampal function [76] and *neuregulin* has effects on prefrontal activity [77]. By better characterising the function of genes implicated in schizophrenia, these studies might inform the development of new treatments designed to target these genes or their products.

Neuroimaging has revealed that some of the brain abnormalities associated with schizophrenia progress as the disorder develops. In particular, there appear to be longitudinal reductions in medial temporal and cingulate cortical volume in the prodromal phase immediately before the onset of schizophrenia [35,78]. This raises the possibility that there is a neuropathological process that is active during the prodromal period. A progressive loss of cortical volume is consistent with the putative involvement of a neurotoxic or neurodegenerative process in schizophrenia [34,35], suggesting that drugs that could block the latter might be effective. PET evidence that presynaptical striatal dopamine function is also perturbed before the onset of schizophrenia [45] suggests that treatment focused on the dopamine system might be useful at this stage, in line with recent evidence that administration of antipsychotics in subjects with prodromal symptoms reduces their risk of later developing schizophrenia [46,47]. Chronic schizophrenia is associated with extensive neuroanatomical, neurocognitive and neurochemical abnormalities. It is therefore unlikely that a single pharmacological agent could correct these if administered after the illness and underlying neuropathology have been established. Drugs that can be used before this point might have a better chance of having disease-modifying, as opposed to symptomatic, effects.

Concluding remarks

The diagnosis and treatment of schizophrenia presents clinicians with several challenges. Neuroimaging techniques have the potential to facilitate diagnosis, help predict which individuals at high risk will later develop the disorder and provide a scientific basis for the development of novel pharmacological approaches. A key challenge to progress in this area is that a core pathophysiological abnormality that underlies schizophrenia has yet to be identified. This might reflect the fact that the clinical category of schizophrenia is heterogenous [79], making it difficult to use functional neuroimaging to identify a pathognomonic abnormality or develop drugs that can treat all features of the disorder. Therefore, future studies might be aided by employing classifications of psychotic disorders that are more homogeneous and closely related to neurobiological abnormalities. For example, positive psychotic symptoms are not present in all patients with schizophrenia, and they also occur in other disorders. However, these symptoms appear to be related to dopamine dysfunction independent of the clinical diagnosis. Future progress will also require the

development of better neuroimaging methods for studying transmitter systems other than dopamine that are implicated in schizophrenia, particularly the glutamate system.

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