Human pharmacology of current and new treatments for schizophrenia

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CHAPTER 1

INTRODUCTION

Background schizophrenia

Schizophrenia, which is in the top ten of most debilitating psychiatric disorders [1], affects up to 1-1.5% of the world population. Usually the symptoms of schizophrenia occur in young adulthood and persist for the entire lifetime [2]. Schizophrenia means a shorter life expectancy, an almost life-long loss of economic productivity and higher health care costs for many patients [3-5]. There is no schizophrenia therapy available today that addresses these issues.

This thesis describes a number of studies related to early drug development for schizophrenia and related disorders. The reader will note the pharmacological diversity of drugs and systems that are dealt with in this thesis. In a way, this reflects the clinical complexity of schizophrenia. According to the Disease State Manual DSM-IV of the American Psychiatric Association [6], the diagnosis of schizophrenia requires the presence for a significant part of one month of at least two of the following diverse symptoms: delusions, hallucinations, disorganized speech, grossly disorganized or catatonic behaviour and negative symptoms. People diagnosed with schizophrenia usually experience a combination of positive symptoms (i.e. hallucinations, delusions, incoherent thoughts), negative symptoms (i.e. apathy, lack of emotion, poor or nonexistent social functioning) and cognitive dysfunction (memory problems, disorganized behaviour, and difficulties planning, concentrating, following instructions or completing tasks) [7-9]. Many researchers include the cognitive deficits with the negative symptoms in the articles they write. Furthermore, many patients suffer from comorbid disorders like depression and anxiety [7]. The most frequently used instrument to measure the signs and symptoms of schizophrenia has been the Positive and Negative Syndrome Scale (PANSS), which in its common form addresses three factors: positive and negative symptoms and general psychopathology [10]. Due to growing knowledge of neurobiology and neuropharmacology of schizophrenia and the increasing recognition of comorbid symptomatology, new ways have been proposed to assess signs and symptoms associated with schizophrenia more accurately. Many publications now identify five symptom categories, which can be roughly divided into positive and negative symptoms, plus disorganization (impaired cognition), excitement, and emotional distress [11-15].

Pathophysiology and etiology

Very little is known about the pathophysiology and etiology of schizophrenia. The situation is complicated by the clinical variability of schizophrenia. It is increasingly suggested that schizophrenia is not a single disease, but a clinical syndrome, perhaps comprising several disease entities [16], and certainly associated with different syndromes like depression and anxiety, in addition to positive, nega-tive and cognitive symptoms. Many hypotheses have been generated in an attempt to explain the pathogenesis and phenomenology of schizophrenia. These can be roughly divided into a neurodevelopmen-tal and neuropharmacological hypothesis [17]. As the neuropharmacological hypothesis will be more relevant for this thesis, this will be discussed in more detail than the neurodevelopmental hypothesis.

Neurodevelopmental hypothesis of schizophrenia

The neurodevelopmental hypothesis of schizophrenia postulates that an environmental insult (e.g. obstetric complications, maternal viral infection, nutritional deficits, psychological experiences) disrupts normal brain maturation, resulting in the emergence of psychosis at puberty or young adulthood [18]. However, a purely environmental origin of schizophrenia is refuted by the strong genetic predisposition, which accounts for 24-80% of the risk of developing the disease (depending on the diagnostic criteria or the use of endophenotypes [17]). The neurodevelopmental hypothesis was therefore later extended to the 'two hit hypothesis', which postulates that an interaction between an early life insult and multiple susceptibility genes is required to cause schizophrenia [19,20]. This hypothesis could be broadened, as Henquet *et al* showed that an environmental factor in a later stage in life (cannabis use), synergistically with genetic predisposition, can also increase the risk of developing psychosis [21-23].

A neurodevelopmental pathogenesis is compatible with the numerous molecular and structural changes that have been found in the brains of schizophrenic patients [24]. Many of the observed changes in gray matter would seem to result from reduced neuropil density (network of dendrites, dendritic spines, axons, and pre- and postsynaptic terminals) and support the suggestion that schizophrenia is a disorder of synaptic connectivity [25,26]. These changes are part of widespread neuroanatomical derangements, which are also reflected by the progressive cerebral (temporal) volume reductions that have repeatedly been found with neuroimaging [27]. Several compounds that target brain growth factors are in early stages of development [28-30]. The currently used drugs are not able to reduce or reverse the structural and neurodegenerative aspects of schizophrenia. However, in some studies indications for secondary structural effects of symptomatic antipsychotics have been found [31,32]. For example, Brennand *et al* showed that loxapine (a registered atypical antipsychotic in the US, structurally related to clozapine) resulted in a statistically significant improvement in neuronal connectivity [26]. If corroborated, these findings illustrate the intricate and reciprocate - though still poorly understood - relationships between psychological, social and biological development of the nervous system in childhood and adolescence.

Neuropharmacological hypotheses of schizophrenia

The pathogenesis of schizophrenia and the pathophysiology of its clinical manifestations have also been related to abnormalities of neurotransmitter systems, which are thought to be the consequences of the underlying neurodevelopmental derangements [33,34]. The most important neuropharmacological hypotheses are based on observations that key symptoms of schizophrenia such as psychotic-like or disorganized states could be either suppressed with or caused by certain drug classes that affect the actions of for example dopamine, serotonine, glutamate, endocannabinoids, GABA (gamma-aminobutyric acid) and acetylcholine.

DOPAMINE

Many different pharmacological systems have been suggested to be implemented in schizophrenia, but the most widely accepted theory regarding the origin of psychotic symptoms is the dopamine hypothesis [35,36]. This hypothesis is based on two observations. First, substances that increase dopamine neurotransmission, such as amphetamine and cocaine, have psychomimetic properties in non-schizophrenics and enhance symptoms in schizophrenia patients [37-39]. And second, the central mechanism of antipsychotic action of even the newer antipsychotics is directed at dopamine D_2 receptors. The first publication of what was later called 'the dopamine receptor hypothesis', reported on the antipsychotic actions of chlorpromazine (introduced in the 50s as the first antipsychotic drug) in a trial with 38 schizophrenic patients [40]. Carlsson and Lindqvist introduced the mechanism of action of antipsychotics by showing that they increased the turnover of monoamines as reflected by increased levels of their metabolites in animals [41]. The dopamine receptor was only identified and firmly linked to antipsychotic response in the 70s [42-45]. It was not until the mid-1990s that imaging studies provided supporting evidence [46-48]. A systematic literature review by De Visser *et al* showed a relationship between D_2 affinity and therapeutic starting dose across a large number of classic and atypical antipsychotic drugs [49].

Changes in dopamine regulation play a major role in both the symptoms and treatment of schizophrenia. In this theory several dopamine tracts are involved (figure 1), which account for the majority of clinical symptoms of schizophrenia and adverse effects of antipsychotic drugs.

Emotional tracts:

1. Mesolimbic pathway (dopamine 2 receptors): begins in the ventral tegmental area (VTA) and connects to the limbic system via the nucleus accumbens (NA), the amygdala, and the hippocampus as well as to the medial prefrontal cortex. Dopamine in the hippocampus is thought to

play a role in new episodic memories and in spatial coding (orientation in space) [50]. Dopamine in the VTA - NA pathway plays a role in the reward system and eating behaviour [51]. The exact role of the mesolimbic dopamine system in the reward system is widely discussed [52]. Three hypotheses have been proposed as explanations for dopamine's function in the reward system: hedonic, learning and motivational salience hypothesis [52]. This hypothesis is discussed in more detail in the next chapter of this introduction.

2. Bulbus olfactorius (not depicted in figure 1 as it is not thought to play an important role in schizophrenia or antipsychotic drug action): involved in olfaction, the perception of odours.

Cognitive tracts:

3. Mesocortical pathway (dopamine 1 receptors): begins in the VTA and connects to the prefrontal cortex. It is thought to be involved in working memory, time orientation, analysis and arguing, planning and mental organisation, and initiative and motivation.

Motor tracts:

4. Nigrostriatal pathway (dopamine 1 receptors): begins in the substantia nigra and projects to the basal ganglia. It is part of a system called the basal ganglia motor loop, and involved in psychomotor and supportive locomotion and facial expression.

Neuroendocrine tracts:

5. Tuberoinfundibular pathway (both dopamine 1 and 2 receptor): begins in the hypothalamus and projects to the posterior pituitary. It is involved in regulation of prolactin secretion from the anterior pituitary gland and metabolic activity.

6. Area postrema (not depicted in figure 1 as it is not thought to play an important role in schizophrenia): controls vomiting through dopamine 2 receptors. Its privileged location in the brain also allows the area postrema to play a vital role in the control of autonomic functions by the central nervous system

MESOLIMBIC DOPAMINE TRACTS At first, it was hypothesized that the mesolimbic dopamine tracts would be hyperactive causing excessive stimulation of D_2 receptors, resulting in positive symptoms. While there is general acceptance of a role for a dopaminergic abnormality in psychosis, what the exact underlying mechanism is, is still a matter of debate. It has been postulated that the mesolimbic dopamine abnormality may be a secondary consequence of another deficit, for example hypo-functionality of the frontal cortex (associated with a reduced glucose utilization and blood flow in the prefrontal cortex, also called cerebral hypofrontality) [53,54], a glutamate deficit [55,56], or a primary neurodevelopmental disorder [34,57]. All these models include a final mesolimbic dopamine dysregulation, but do not specify how this leads to symptoms. The so called aberrant salience hypothesis of Kapur may provide a link between the biological dysfunction of the dopamine system and the symptomatic expression of psychosis [58]. To understand this hypothesis, it is necessary to know one of the roles of dopamine on the mesolimbic pathway (earlier described as 'initiative and motivation' as functions of the mesolimbic tract), called the motivational salience hypothesis [51,59-61]. According to this hypothesis, dopamine mediates the conversion of the neural representation of an external stimulus from a neutral bit of information into an attractive or aversive entity [51]. Thus, dopamine, which under normal conditions is a mediator of contextually relevant saliences, in the psychotic state becomes a creator of aberrant saliences. According to the idea of salience attenuation, antipsychotics do not primarily change thoughts or ideas; instead, they provide a neurochemical milieu in which new aberrant saliences are less likely to form and previously aberrant saliences are more likely to extinguish [62]. They do not remove the core content of the symptom, but rather the degree to which the symptoms occupy the mind, distress the patient, and drive action [63]. In neurochemical terms this means that antipsychotics are seen as blocking the expression of abnormal dopaminergic transmission, but they do not fundamentally alter the dopaminergic dysregulation [64,65]. This explains why delusions and hallucinations do not immediately resolve when treatment is introduced, but do lose their emotional significance and pervasive

character. It is only after some weeks of treatment that the fundamental content of the delusions and hallucinations is deconstructed and recedes (entirely for some) from awareness[66,67]. The detailed mechanism behind this concept is unknown, but it fits well for the phenomenon of relapse, exacerbation and recurrence of psychosis in schizophrenia[68].

MESOCORTICAL DOPAMINE TRACTS The classical dopamine hypothesis of schizophrenia thus postulates that an excess of dopamine subcortically (as a consequence of hyperactivity of the mesolimbic pathway) is associated with positive symptoms of schizophrenia. About 20 years ago, it was proposed that the negative symptoms and cognitive impairment, commonly found in schizophrenia, could be associated with a lack of dopamine in the prefrontal cortex [69]. This idea was raised, because dopamine depletion in the prefrontal cortex produced cognitive impairment in animals [70]. Functional imaging studies [71,72] and postmortem studies of the brains of schizophrenic patients [73-75] demonstrated a range of abnormalities in cortical areas within the working memory network, including (most consistently) the dorsolateral prefrontal cortex, cingulate, and temporal cortices. The overall function of a cortical network presumably relies on the competence of both local information processing within specific local circuits and axonal connections between local circuits and distant cortical areas. Therefore, a deficit of a single region (for example in the dorsolateral prefrontal cortex) could conceivably have functional consequences in the working memory network [72].

MESOLIMBIC AND MESOCORTICAL IMBALANCE Combining the mesolimbic and mesocortical theories, the current dopamine hypothesis suggests an imbalance between mesocortical and mesolimbic dopaminergic systems [53,76]. Several hypotheses have been published on how the overactivity and underactivity in these different regions can co-exist at the same time and whether these are linked. There is an ongoing debate whether subcortical dopamine dysregulation (mesolimbic) is a primary phenomenon, or is rather associated with prefrontal cortical dysfunction (mesocortical) [77-80].

Neuroimaging studies have contributed to a better understanding of the pathophysiology of schizophrenia and give strong evidence for the 'combined mesolimbic and mesocortical dopamine hypothesis' of [76,81,81-83]. Davis *et al* postulates that a deficit of dopamine in the prefrontal cortex results in hypostimulation of D_1 receptors (leading to cognitive deficit symptoms [84,85]), the predominant dopamine receptor subtype in this area [53]. In addition, these alterations feed into each other as it has been shown that cortical dopamine has an inhibitory effect on subcortical dopamine. This deficit in cortical dopamine may itself contribute to excess in subcortical dopamine activity in mesolimbic dopamine neurons (inducing positive symptoms) [86]. The hypothesis of Abi-Dargham is similar to that of Davis *et al*, but slightly more detailed. It makes use of an excitatory (glutamatergic) and inhibitory (GABAergic) system, which both control the dopamine neurons in the VTA to explain a simultaneously-induced imbalance of the two systems [76].

GENETIC VULNERABILITY AND DOPAMINE Although susceptibility to schizophrenia is largely explained by genetic variation, demonstrated by family, twin and adoption studies [87], schizophrenia is a complex disorder, not simply defined by several major genes, but rather evolving from addition or potentiation of a specific cluster of genes, which subsequently determines the genetic vulnerability of an individual. Linkage and association studies suggest that genetic factors increase the vulnerability to the disease, but that penetration is modulated by different triggering factors and environmental influences. Over the past years, a large number of genes or polymorphisms have been evaluated, which could in some way be related to pharmacological systems involved in schizophrenia. Several studies and meta-analyses point at the potential involvement of the gene for dopamine D_2 receptors (DRD2) [88,89]. However, the relationship between polymorphisms and schizophrenia is complex and makes it impossible to draw strong, direct conclusions as dopamine availability and brain functioning are not linearly related [90]. Catechol-O-methyl transferase (COMT), a catabolic enzyme involved in the degradation of particularly dopamine [91,92], has been shown

to be critical for prefrontal dopamine flux as well as prefrontal cortex-dependent cognition and activation [93]. Several COMT polymorphisms substantially influence the activity of the enzyme [90,93]. Except for COMT, studies looking for links between polymorphisms and schizophrenia have mostly been negative or ambiguous [81,94-97].

In summary, the dopamine hypothesis has been and still is the currently most widely accepted theory of schizophrenia. Even though it has been accepted that changes in dopamine are playing a role in schizophrenia, it still remains a matter of debate whether dopamine is the primary or only cause of these derangements.

SEROTONIN

Serotonin was discovered in the late 1940s, and the first serotonin (5-HT) receptors were identified shortly thereafter [98]. In the 1950' and 60's, the partial 5-HT_{2A} agonist lysergic acid diethylamide (LSD) was extensively used and even registered for some time (as Delysid¨), for its hallucinatory effects and as a tool to investigate or experience psychotic-like states. Interest in the role of serotonin for the treatment of schizophrenia was further enhanced by the discovery of the efficacy of clozapine in treatment resistant schizophrenia [99]. The use of this drug is thwarted by the occurrence of rare but serious adverse events like bone marrow suppression, but it is an effective antipsychotic medication that causes fewer extrapyramidal and cognitive side effects than other antipsychotics [100]. Clozapine has a relatively low affinity for the D_2 receptor, but it affects a range of other targets, including 5-HT_2 receptors [101]. Clozapine's specific properties have contributed to the serotonin-dopamine hypothesis, which states that a certain ratio of serotonin 5-HT_2 to dopamine D_2 affinity is the most critical mechanism behind the atypical antipsychotic action. For a long period, this has been the most widely accepted basis for atypicality [102,103]. The thought behind this hypothesis is that by blocking the presynaptic 5-HT_{2A} receptors in the nigrostriatal and mesocortical tracts, the net effect of a decrease in dopamine neurotransmission (and therefore the associated side effects seen with chronic antipsychotic therapy in these tracts) would be reduced.

However, this hypothesis has frequently been criticized as 'pure' 5-HT_{2A} antagonists have never shown antipsychotic properties.

Although the interest in the 5-HT_{2A} antagonists has decreased, there is increasing evidence that 5-HT_6 receptors are involved in cognition and learning, and that they might play a role in convulsive disorders and appetite control [98]. It has been hypothesized that clozapine's relatively high affinity for 5-HT_6 receptors might explain some of its beneficial cognitive effects in schizophrenia [104,105]. In animals 5-HT_6 receptor antagonists showed improvements in several cognition models [106-109], although contradictive results were reported as well [110,111]. Several reports suggest a potential therapeutic role of 5-HT_6 receptor antagonists in the treatment of cognitive dysfunction. Interestingly, these agents seem to be effective in syndromes associated with cholinergic hypofunction [112].

Summarizing, 5-HT_{2A} antagonism by itself has never been persuasively shown to have antipsychotic properties, despite clear evidence for psychomimetic effects of 5-HT_{2A} activation. However, 5-HT_6 antagonism might be promising in the treatment of cognitive impairment in schizophrenia.

GLUTAMATE

In the late 50s it was hypothesized that hypofunction of the glutamatergic system, and specifically of the N-methyl-D-aspartate (NMDA) receptor, contributed to the pathophysiology of schizophrenia [113,114]. This was based on effects of non-competitive antagonists of the N-methyl-D-aspartate (NMDA) glutamate receptor (e.g. ketamine and phencyclidine), which exacerbated symptoms in schizophrenic patients and induce psychotic symptoms in healthy men [115]. The effects were considered to better resemble schizophrenia than those induced by dopamine activation or partial 5-HT_2 agonism: not only positive, but also negative symptoms and cognitive deficits were shown after administering these compounds [115,116]. Furthermore, reciprocal synaptic relationships between forebrain dopaminergic projections and glutamatergic systems have been described and therefore dysfunction of glutamatergic neuronal

systems would not be inconsistent with the dopamine hypothesis of schizophrenia [113]. Olney and Farber conjectured that dopamine receptors inhibit glutamate release [117,118]. A primary defect in the dopamine system that causes dopamine hyperactivity could result in excessive suppression of glutamate release at NMDA receptors, with consequent hypofunction of the NMDA receptor system, which could be the basis for schizophrenic symptoms [113]. It was stated that this theory would fill some of the gaps of the dopamine hypothesis.

Receptors for glutamate are divided into two broad families: 1) ionotropic receptors, which are differentiated based upon sensitivity to the synthetic glutamate derivatives NMDA, AMPA and kainate and 2) metabotropic receptors, which are G protein coupled and mediate longer-term neuromodulatory effects of glutamate [119]. In addition to the recognition site for glutamate, NMDA receptors contain allosteric modulatory sites for glycine, which affects channel open time and desensitization rate in the presence of glutamate. AMPA and kainate receptors mediate the majority of glutamate in the brain. AMPA receptors work heavily in concert with NMDA receptors. Metabotropic receptors, which regulate glutamatergic neurotransmission both pre- and post-synaptically, may serve as an alternative pharmacological target for the treatment of schizophrenia [119].

Summarizing, hypofunction of the glutamatergic system is thought to contribute to the development of schizophrenia. Furthermore, it would not be inconsistent with the dopamine hypothesis.

ENDOCANNABINOIDS

Many articles have been published about the relationship between the use of marijuana and schizophrenia and there is little doubt that cannabis can lead to clinical psychosis, particularly in susceptible individuals and/or after heavy use [21,22]. It is believed that many of the pharmacological effects of marijuana are mediated through the specific endogenous receptors CB_1 and CB_2, and it has been proposed that dysfunction of the endogenous cannabinoid system may play a role in the production of at least some of the symptoms of schizophrenia [120].The finding that levels of two endogenous cannabinoids were increased in the cerebrospinal fluid of schizophrenic patients seems consistent with this hypothesis. However, the direct contribution of endocannabinoid systems in the pathophysiology of the psychotic symptoms of schizophrenia are still unclear [121].

The evidence for pharmacological relationships between endocannabinoid and dopamine systems is increasing [122,123] and psychotic symptoms have consistently been related to increased dopamine function in the striatum [43]. In a study of Fernandez-Ruiz, the literature addressing the cannabinoid-dopamine interactions was reviewed [124]. The CB_1 receptor in particular functions as a retrograde signalling system in many synapses within the CNS, particularly in GABAergic and glutamatergic synapses. They also play a modulatory function on dopamine (DA) transmission, although CB_1 receptors do not appear to be located in dopaminergic terminals in the major brain regions receiving dopaminergic innervations (e.g. the caudate-putamen and the nucleus accumbens or prefrontal cortex). Therefore, the effects of cannabinoids on DA transmission and DA-related behaviours seem generally indirect and exerted through the modulation of GABA and glutamate inputs received by dopaminergic neurons. Recent evidence however suggests a direct interaction between cannabinoid and dopaminergic pathways [125,126]. Cannabinoids have an important influence on various DA-related neurobiological processes (e.g. control of movement, motivation/reward) and pathologies (schizophrenia, basal ganglia disorders, and drug addiction) [124]. Bossong *et al* have shown that striatal dopamine release is increased by an acute dose of Δ9-tetrahydrobannabinol (THC), the most important $CB_{1/2}$-partial agonist from marijuana, which may explain how cannabis contributes to the development and pathophysiology of schizophrenia [127]. In chapter 8 a study is presented which supports the notion that psychotic-like effects induced by THC are mediated by dopaminergic systems, but that other systems also seem to be involved in the 'feeling high' effects. Additionally, the clear reductions of psychotic-like symptoms by a clinically relevant dose of haloperidol suggest that THC administration may be a useful pharmacological cannabinoid model for psychotic effects in healthy

volunteers. A similar study was recently performed by Kleinloog *et al* using olanzapine instead of haloperidol. They found similar results, confirming the use of this practical psychosis model for the currently used antipsychotics. It would be interesting to investigate whether this model can also be used for compounds with other mechanisms investigated for the treatment of schizophrenia.

In summary, relationships between cannabis and psychosis are increasingly clear. There is increasing evidence for a pharmacological relation between (endo)cannabinoid and dopamine systems. However, it is uncertain whether endocannabinoids contribute to the pathophysiology of schizophrenia.

GAMMA-AMINOBUTYRIC ACID (GABA)

The GABA$_{(A)}$ergic (gamma-aminobutyric acid) system has been implicated in the pathophysiology of schizophrenia, both for positive, negative [128,129] and cognitive symptoms [130]. GABA$_{(A)}$ergic interneurons are a core component of the corticolimbic circuitry and modulate the mesolimbic and mesocortical dopaminergic system in a complex way. A growing body of evidence suggests that a malfunction in cortical GABAergic transmission resulting in a disturbance in cortical network activity is a critical factor underlying schizophrenia [131]. The exact role of GABAergic systems in the pathophysiology of schizophrenia is still unclear and in part controversial.

GABA$_{(A)}$ agonists have shown beneficial effects on positive, and to a lesser degree, negative symptoms in some schizophrenic patients [129]. On the other hand, some GABA$_{(A)}$ agonists like zolpidem can induce florid hallucinogenic effects [132,133]. Moreover, GABA$_{(A)}$ agonists such as benzodiazepines, although widely used as sleep aids in schizophrenia, can impair, rather than correct, sleep architecture and cognition [134].

GABA$_{(B)}$ agonists, may be a better option for sleep problems in schizophrenia [134]. Whether GABA$_{(B)}$ agonists have a good or deleterious effect on the positive and negative symptoms has not been sorted out yet. These apparently contradictory reports are at least partly due to two important factors. Firstly, many schizophrenic patients suffer from co-morbid anxiety and disordered sleep, which can have profound effects on the intensity of hallucinations, delusions, and on cognitive organization. Benzodiazepines and other GABA$_{(A)}$ agonists are well-known for their anxiolytic and sleep inducing effects, which during short term treatment may secondarily improve sleep and decrease paranoia. At the same time however, GABA$_{(A)}$ agonists are equally well-known for their detrimental effects on memory and attention, particularly at higher doses and in cognitively vulnerable subjects, and there is no reason to suspect that this would be different in schizophrenia. These two aspects of generalized GABA$_{(A)}$-activation thwart the non-discriminant use of benzodiazepines in schizophrenia, although these treatments can be quite useful in case of pronounced anxiety, restlessness or insomnia. Non-selective GABA$_{(A)}$ receptor inverse agonists (with a negative efficacy at all a subunits) have been shown to improve alertness and memory in experimental animals, but such drugs can also cause anxiety and seizures, and have therefore not been studied extensively in humans [130]. Secondly, GABA$_{(A)}$-receptors are composed of different subunits, which have increasingly been associated with different CNS-functions. GABA$_{(A)}$ agonists containing alpha$_1$ subunits are associated with alertness and sedation. GABA$_{(A)}$ agonists with a high affinity for these subtypes have been developed as potent hypnotics. Particularly these so-called z-hypnotics (including zolpidem, mentioned earlier) can sometimes cause hallucinations, which has been attributed to a dissociated wake-sleep transition [132]. GABA$_{(A)}$ receptors containing alpha$_2$ and alpha$_3$ subunits are linked to anxiety and anxiolysis. Subtype selective alpha$_{2,3}$ agonists are in development for anxiety disorders with an improved side effect profile. These drugs have not been studied in schizophrenia [128] but could be useful as add-on treatments for hallucinations or paranoid delusions or other co-morbid anxieties. Alpha$_4$ containing GABA$_{(A)}$ receptors are located extrasynaptically throughout the brain. Their physiological role is uncertain. GABA$_{(A)}$ receptors containing alpha$_5$ subunits have been associated with memory. Consequently, alpha$_5$-selective inverse agonists have been in development as memory-enhancers for various cognitive disorders, but they have not been examined in schizophrenia.

In conclusion, specific GABA(A)ergic systems (or specific receptor subtypes of the GABA system) may be relevant for various functional abnormalities in schizophrenia, which opens possibilities for potential novel treatments for specific clinical features of this disease. However, schizophrenia is a complex disorder with a multitude of pharmacological derangements, and the role of GABAergic systems is still largely unclear. At present, the development of GABA-ergic drugs is mainly directed at more restricted clinical syndromes, with better established links to the different GABA-systems and their functional characteristics, such as anxiety and memory impairment.

ACETYLCHOLINE

The cholinergic system (and acetylcholine) is thought to play a role in the symptoms of cognitive dysfunction in schizophrenia [129,135,136]. Cognitive impairments observed in schizophrenia in many aspects resemble those that occur in healthy subjects following administration of scopolamine [137,138]. Scopolamine has been applied as a disease model for dementia [139] and for memory impairment in schizophrenia [137]. Although the scopolamine model does not capture the complexity of cognitive decline in schizophrenia (or Alzheimer's disease for that matter), it has become the most frequently used model for studies of cognitive impairment in experimental animals and healthy volunteers [139-142]. Scopolamine-induced memory impairments in animals show similarities to those in humans [143,144]. It is thought that there is an interaction between cholinergic and glutamatergic systems on cognitive function although the exact mechanism behind this interaction has not been resolved [145-147]. In chapter 5 and 6 of this thesis, the scopolamine model was used in healthy volunteers to study the effects of two new glycine reuptake inhibitors. In chapter 7 the pharmacokinetic-pharmacodynamic relationships were investigated.

The evidence that abnormalities in the cholinergic system may play a role in other symptoms of schizophrenia is still marginal and will therefore not be discussed further in this introduction.

NEUROPHARMACOLOGICAL COMBINATION THEORY

The different neuropharmacological theories are not mutually exclusive and can be combined to a 'neuropharmacological combination model' (partially adapted from Stahl [11]). The relationship between dopaminergic, serotonergic, glutamatergic and GABAergic tracts in people without schizophrenia could be simplified as shown in figure 2. The endocannabinoid system has not been included in the picture, since the position of endocannabinoid systems is still unclear and could in fact overlap with several of the other neurotransmitter pathways, by direct or indirect interactions with dopamine and GABA or glutamate [124]. This is also the case for acetylcholine.

In the VTA the cortical brainstem glutamate projection is linked to the mesolimbic dopamine pathway through a GABA interneuron. Stimulation of interneuron NMDA receptors by glutamate induces GABA release. This inhibits dopamine release from the mesolimbic dopamine pathway, which communicates with the parahippocampal gyrus, linked with the sensory associative cortex in both ways, resulting in 'recognition' of sensory information. The mesolimbic pathway also communicates with the amygdala, which also links to the sensory associative cortex, resulting in the realization whether something is 'safe' or whether it is a 'threat'. This can be explained by the motivational salience hypothesis. According to this hypothesis, dopamine can be a mediator of contextually aberrant saliences as was explained before in this introduction [51].

In this relatively simple integrated neuropharmacological model of schizophrenia, NMDA receptors in the cortical brainstem glutamate projection would be hypoactive, resulting in disinhibition of the mesolimbic dopamine pathway, leading to hyperactivity of this pathway and eventually to positive symptoms (figure 3).

The cortical brainstem glutamate projection also communicates directly with the mesocortical dopamine pathway in the VTA, normally causing tonic excitation. In schizophrenia, hypoactivity of NMDA receptors in cortical brainstem glutamate projections leads to a loss of tonic excitation, and mesocortical dopamine pathways become hypoactive, which could contribute to cognitive, negative and affective symptoms (figure 3).

Pharmacological treatment
Currently used antipsychotics

Almost all currently available antipsychotic drugs are based on the dopamine hypothesis, and although many drugs have been designed to affect or avoid certain other specific pharmacological targets, all registered antipsychotics antagonize the dopamine D_2 receptor [49]. Several publications have clearly reviewed these drugs (see for example [148,149]) and therefore only a brief summary will be given.

The current pharmacological treatments can be divided in two groups: 1) first-generation antipsychotics (FGAs), also called classic or conventional antipsychotics or neuroleptics; and 2) second-generation antipsychotics (SGAs), also called atypical or nonconventional antipsychotics. The FGAs (phenothiazines, butyrophenones, thioxanthines) have been the main therapy until the introduction of SGAs in the early 1990s. Although it is not completely clear what differentiates FGAs from SGAs (as discussed in the next chapter of this introduction), the term 'atypicality' refers to drugs that have at least equal antipsychotic efficacy compared to conventional drugs, with less extrapyramidal side effects (EPS) (acute dystonic reactions, akathisia, parkinsonism, tardive dyskinesia) and/or prolactin elevation [148]. The extrapyramidal syndromes such as medication-induced parkinsonism and dystonias are caused by dopamine antagonism in the nigrostriatal tract. Low dopamine in the tuberoinfundibular tract leads to prolactin release, which in some patients leads to gynecomastia.

The high risk of the possibly irreversible movement disorder tardive dyskinesia, and the high rates of acute EPS were the major reason for psychiatrists to switch patients to SGAs when these became available. Many studies have been performed that compare FGAs to SGAs or SGAs to each other. These studies, however, were often performed in patients with acute exacerbation, did not include treatment-resistant patients, were relatively short and did not take into account sufficient efficacy and safety parameters. Moreover, these studies were usually sponsored by the manufacturer of the new antipsychotic and often included a higher than necessary dose of the comparator FGA [149,149]. In the CATIE (Clinical Antipsychotic Trials of Intervention Effectiveness) trial, in which discontinuation of treatment was the main endpoint, a difference between the studied SGAs and FGA was found [150]. Olanzapine was the most effective in terms of lower rates of discontinuation, but the efficacy of the FGA perphenazine appeared similar to that of the other SGAs (quetiapine, risperidone, and ziprasidone). However, olanzapine was associated with greater weight gain and increases in measures of glucose and lipid metabolism. Several other meta-analyses did not lead to a definitive answer to the question whether FGAs and SGAs differ in efficacy [150-158].

The only exception seems to be clozapine, which has repeatedly been effective for treating both positive and negative symptoms in treatment resistant schizophrenia [100], but may cause severe side effects such as agranulocytosis that limit its use [159-161]. The beneficial pharmacological properties of clozapine are unknown [162], and many different hypotheses for the superior effectiveness in treatment resistant schizophrenia have been published.

Many have claimed that the SGAs are more efficacious than FGAs in reducing negative symptoms (e.g., lack of emotion, interest, and expression), possibly due to the absence of extrapyramidal symptoms [163] or other secondary causes of negative symptoms (e.g. depression) rather than to direct therapeutic effects [164]. However, it should be noted that this is difficult to assess, because it is hard to distinguish secondary negative symptoms (due to some features of EPSs caused by antipsychotics [163] or exacerbation of mesocortical hypodopaminergic function by D_2 antagonists) from primary negative symptoms of the disease. Lehman *et al* conclude in the second edition of the American Psychiatric Association (APA) Practice Guideline for the treatment of patients with schizophrenia that at this moment there is no effective treatment for primary negative symptoms [165].

Comparable to negative symptoms, it is unknown whether there is a difference in effects on cognitive impairment and mood disturbance [166,167]. The ability of atypical agents to prevent relapse and their effects on social and vocational functioning, quality of life, long-term

outcome, and the caregivers' burden have been incompletely explored [156,163,168]. Similar to negative symptoms, cognitive dysfunction may be secondary to other factors such as EPS, anticholinergic effects and sedation or the underlying disease.

The superior tolerability of the SGAs with regard to EPS seems proven [151,152,152,158,169]. They do, however produce other side effects, of which weight gain [170,171] in combination with an altered glucose, cholesterol and lipid metabolism [172-174] seem to be the most worrisome. Besides the fact that this can adversely affect quality of life and medication adherence [175], weight gain and metabolic abnormalities are important risk factors for premature cardiovascular morbidity and mortality [176]. It is unclear whether the glucose effects are secondary, independent or causative to weight gain. Also, to date, research has been inconclusive whether antipsychotics increase weight via increased appetite and food intake, decreased activity or decreased metabolism. Which pharmacodynamic receptor is responsible for this weight gain and metabolic changes, has yet to be determined [177]. Taken together, the available preclinical and human data indicate that no single neurotransmitter system is responsible for antipsychotic-related weight gain. While rodent and indirect human evidence links the weight gain potential of antipsychotics to histamine H_1 blockade [178], studies also implement other neurotransmitter systems, such as dopamine D_2 blockade, 5-HT_{2C} blockade and interactions with central or peripheral hormones and peptides involved in energy homeostasis [177]. These results are further supported by evidence of an interaction between histamine H_1 and dopamine D_2 blockade [179], genetic data [180] and by the fact that antipsychotics without relevant antihistaminergic activity, such as aripiprazole, amisulpride and haloperidol, have clearly documented weight gain potential, especially in antipsychotic-naive and first episode patients [181].

There are differences in weight gain between the atypical antipsychotics [177]. The risk is highest after the use of clozapine or olanzapine and lower (but still present) after risperidone, quetiapine and aripiprazole.

Another known adverse effect of antipsychotics is cognitive dysfunction. A growing body of evidence from such animal studies indicates that several antipsychotics, including both SGAs and FGAs (if administered for sufficient periods of time), can be associated with impairments in memory-related task performance, as well as alterations in central cholinergic function [182]. It is not completely clarified how this can be explained pharmacologically, but the importance of cholinergic receptor to information processing and cognitive function is thought to play a role [182].

Pharmacological theories for differences between FGAs and SGAs

Many hypotheses have been generated for the pharmacological difference between FGAs and SGAs. According to Kapur and Seeman all efforts to produce antipsychotic action without D_2 blockade have been unsuccessful [183]. Kapur and Seeman proposed that a low affinity at the D_2 receptor in and of itself, is sufficient for producing atypical antipsychotic activity. Affinity (Kd) is defined as the ratio of k_{OFF}/k_{ON} (the rate at which the drug moves off and binds to the receptor). It has been found that koff is the most important determinant of how drugs and dopamine compete [183]. The faster koff, the more quickly the drug will release from the receptor after an endogenous dopamine surge. The slower koff, the slower the drug responds to changes in endogenous dopamine. Antipsychotics with a fast koff under clinical conditions give rise to a fast-on, fast-turnover and fast-off blockade of D_2 receptors. The fast dissociation hypothesis suggests that the combination of a fast koff at the molecular level and transient D_2 occupancy at the system level is sufficient to provide an atypical antipsychotic effect. Kapur and Seeman propose that drugs with fast dissociation when used in doses that lead to appropriately high D_2 blockade modulate the dopamine system in a manner that allows for a better accommodation to changes in physiological dopamine transmission, permitting an antipsychotic effect without motor side effects, prolactin elevation, or anhedonia and other

secondary negative symptoms, and that this leads to what is currently called the atypical antipsychotic effect. Traditional antipsychotics (e.g. haloperidol and chlorpromazine) bind tightly to the dopamine D_2 receptor and slowly dissociate from the D_2 receptor in vitro or in vivo [184]. The atypical antipsychotic drugs (e.g. quetiapine, clozapine, paliperidone, and amisulpride) show rapid dissociation times from the cloned D_2 receptor [185,186], and clinical dissociation times of hours, thus minimizing clinical side effects.

Aripiprazole is a novel antipsychotic agent that is also considered atypical, but with a slightly different mechanism of action. It is a partial agonist for the dopamine and 5-HT_{1A} receptor and an antagonist for the 5-HT_{2A} receptor.

Another hypothesis for differences in FGAs and SGAs is the '5-HT_2 hypothesis'. Since all antipsychotics block D_2 receptors, it has often been thought that atypical antipsychotics must differ due to a separate receptor mechanism. However, high 5-HT_2 occupancy (or a high 5-HT_2/D_2 ratio) seems neither necessary nor sufficient for the atypical antipsychotic effect [183]. Although this hypothesis could be a relatively easy way to explain the differences between atypical and typical antipsychotics, there are several arguments against this theory. One argument is the absence of an atypical clinical profile of antipsychotic effects, after addition of a selective 5-HT_2 antagonist to treatment with an ongoing D_2 antagonist [36]. Many 'atypical' antipsychotics have also been developed because of their 5-HT_2-inhibiting properties, but selective 5-HT_2 antagonists that lack antidopaminergic activity (such as ritanserin) have failed to show a reliable antipsychotic effect [36]. Second, several typical antipsychotics have a high affinity for 5-HT_2 receptors, and some lose 'atypicality' at high doses, which does not support this theory [187]. Furthermore, the relative freedom from extrapyramidal symptoms of the atypical antipsychotics is not related to their 5-HT_2/D_2 ratio's [188] . Also, this ratio is not associated with the therapeutic doses of different classic and atypical antipsychotic drugs, contrary to D_2-affinity [49,189]. Other less well accepted hypotheses for the difference between typical and atypical antipsychotics will not be discussed in this introduction [65].

Past drug development

The majority of currently approved pharmacological agents target psychotic symptoms as their primary effects, and are largely similar in efficacy and effectiveness (except for clozapine in drug-resistant schizophrenia). Each new atypical drug has its own individual side-effect profile and should therefore be individually evaluated for safety. Although currently used antipsychotics can reduce core psychotic symptoms and delay symptom exacerbations very effectively in many patients, schizophrenia has remained a chronic illness with substantial functional impairments, with limited therapeutic developments. The introduction of the atypical antipsychotics has provided a larger and more diverse armamentarium of treatment options, but companies have mainly focussed on alterations of existing medications and on gaining approval on new indications for already marketed drugs [190]. Although many new mechanisms of action have been proposed, several of which have already been examined for a number of decades, these have not led to concrete new treatment strategies or novel drug registrations for schizophrenia. Therapeutic innovations since the discovery of chlorpromazine were mainly limited to pharmacological modifications of the receptor specificity, affinity and intrinsic activity. In general, efforts to discover and develop new drugs have been relatively unsuccessful compared with other disease areas [191-193], and a sceptic could argue that drug development for schizophrenia has not progressed appreciably since the serendipitous discovery of 'chemical lobotomy' with chlorpromazine in the early '50s [194]. Agid *et al* mentioned several reasons that could underlie this lack of success [191]. Perhaps the most important reason is that psychiatry has a diagnostic and classification system that is not based on etiology, neurobiology, epidemiology, genetics or response to medications, but rather on a constellation of signs and symptoms [193]. As with many other neuropsychiatric conditions, schizophrenia research and drug development are also thwarted by the complexity of the CNS, lack of a defined pathology, problematic tissue accessibility, co-morbidity, absence of good animal models, and the daunting fact that

the complexity of behaviour is not simply the sum of its constituents [191].

Another important reason for the lack of progress in development of schizophrenia drug therapy is the absence of adequate animal models to allow determination of clinical efficacy, which therefore often only gets fully established after the drug is widely prescribed on the market. Current preclinical models for schizophrenia are quite effective at predicting whether a candidate molecule will have 'atypical' properties. However, they are less able to make a prognosis for overall efficacy, and completely unable to predict greater efficacy compared to currently available antipsychotics [192]. Moreover, in terms of the negative and cognitive symptom domains in schizophrenia, none of the commonly used animal models are highly predictive, and although it is expected that preclinical memory models will be useful for forecasting the ability to enhance cognition [190], this has not yet been validated in practice. As validity of most currently used animal models is essentially limited to the dopamine hypothesis, these models favour development of (even more) antidopaminergic antipsychotics, but their predictive value for treatments with entirely different mechanisms of action remains to be established. Another disadvantage of using small laboratory animals as a schizophrenia model is that they lack the complex pre-frontal cortical networks that are responsible for many of the clinical manifestations of schizophrenia and psychosis. Additionally (or consequently), the most relevant (clinical) symptoms such as hallucinations and delusions are difficult to assess or measure objectively in animals. Although these limitations are generally acknowledged, current animal models continue to be used because there are no alternative models available. A more rational approach would be to employ human models for the pharmacological, cognitive and antipsychotic effects of putative novel drugs for schizophrenia. Investigating (new) compounds in healthy subjects in an earlier stage of development will increase the predictive value for (new) compounds and will provide reliable information to the development program at the earliest possible time and reduce exposure to vulnerable populations (i.e. schizophrenia patients) [195]. This topic is dealt with in chapters 7 and 8.

New drug development

Although most new pharmacological approaches to schizophrenia are merely symptomatic, they may still cause relevant improvements in patients. Considering the complexity and the multidimensional characteristics of schizophrenia, it is likely that newly developed medications will increasingly be introduced as parts of a polytherapeutic strategy. Each patient will be treated with an individualized drug combination, aimed at his or her personal multidimensional disease profile [190]. This is also called the 'magic shotguns' approach [196]. This competes with the strategy of the 'magic bullet', that still seems to be favoured by the pharmaceutical industry and to be expected by the society. In this strategy a single perfect drug will cure the disease without side effects. Progressions made with polypharmacy are slower than with 'magic bullets', because of the many complexities of dose determinations of combined treatments and interactions, patient selection, and problems associated with patents and ownership. On the other hand, polypharmacy shows close resemblance to clinical practice, where most patients with chronic psychiatric conditions ultimately receive drug combinations that have been individually selected and optimized, targeted at specific clinical problems, and guided by gradual dose escalations and regular assessments of therapeutic and adverse effects.

Gray gives a good impression of the many pharmacological targets which are under investigation for the development of new drugs for schizophrenia [190] and for cognitive deficits specifically in schizophrenia [197]. The increasing awareness that schizophrenia affects different neuropsychological domains such as cognition, mood and social functioning, has fuelled the notion that other systems may also be involved. Thus, drugs that are primarily in development for dementia (e.g. cholinergic or serotonergic enhancers) or depression (like monoaminergic or vasopressinergic agents) are often also explored as potential treatments for abnormal affection, cognition deficits and social functioning in schizophrenia. In more recent years, psychotic symptoms with other drugs or diseases, animal models and other research findings

have also implicated other neuropharmacological systems, such as GABA, endocannabinoids and glucocorticoids [192]. So far, to our knowledge, many of these strategies have been abandoned.

Since 2008, about around 2600 compounds are in development for the treatment of schizophrenia (source: Prous Science Integrity, http://integrity.prous.com/integrity/servlet/xmlxsl/). These experimental compounds show a remarkable pharmacological diversity, with 210 more or less distinct mechanisms of action. Only roughly one third of the compounds is based on known mechanisms of action or on derivatives of currently existing antipsychotics. Frequently reported mechanisms are glycine-1 reuptake inhibition (203 times), dopamine D_2 antagonism or partial agonism (91), 5-HT_{2A} antagonism (70), tachykinin NK_3 antagonism (62) and 5-HT_6 antagonists (60). In the following paragraphs, several active drug development programs targeted at different pharmacological receptors are discussed, which are also dealt with in other chapters of this thesis.

DOPAMINERGIC STRATEGIES

Virtually all currently available antipsychotic drugs are mainly aimed at positive symptoms, by reducing the excessive mesolimbic parahippocampal dopaminergic tone that seems to be an essential element of psychotic manifestations. At the same time, such treatments also reduce other dopaminergic pathways that do not function excessively and are sometimes even less active in patients. Such pathways are involved in important physiological functions like extrapyramidal regulations, mesolimbic reward system, mesocortical cognitive processes, and tuberoinfundibular metabolic and neuroendocrine activity. These mechanisms are responsible for significant adverse effects of antidopaminergic drugs that contribute to poor compliance and cognitive blunting. In recent years, due to new insights in pathogenesis and pathophysiology of schizophrenia, new pharmacological targets and corresponding compounds have been developed, as for example reviewed by Gray *et al* [190,197]. Some new dopaminergic treatments are designed to modulate rather than simply antagonize the activity of dopamine in the brain. Aripiprazole for instance is a partial D_2 agonist, designed to reduce hyperactive dopaminergic functions (involved in positive symptoms) and to activate reduced dopamine tone in mesocortical (cognitive) areas. Other more indirect strategies are currently investigated. Based on the fast dissociation theory of atypicality described earlier, new agents are now in development that dissociate faster from the dopamine receptor [183]. An example is the centrally acting dopamine D_2 receptor antagonist JNJ37822681, which combines specificity for the dopamine D_2 receptor and a fast rate of dissociation from this receptor [198]. Another indirect strategy is targeted at neuropeptides that modify the activity of monoaminergic neurotransmitters. Tachykinins for instance activate neurokinin NK3 receptors that facilitate the release of the biogenic amines dopamine, serotonin and norepinephrine. This opens the possibility that antagonism of NK-receptor reduces the excitatory activation (or hyperactivity) of some or all of these principal systems, without affecting normal dopaminergic baseline activity so much. Various NK antagonists are in development for different psychiatric disorders[199]. In chapter 3, the effects of the neurokinin $(NK)_3$ antagonist talnetant (SB223412) were investigated in healthy volunteers.

SEROTONERGIC STRATEGIES

Although there is no definite proof for the earlier mentioned serotonin-dopamine hypothesis (in which the 5-HT_2 receptor is involved), there are theoretical grounds for investigating other subtypes of the serotonin receptor. Several serotonergic strategies are outlined by Gray *et al* [190,197]. Glennon *et al* have reviewed three possible new receptors which may be implicated in schizophrenia: 5-HT_5, 5-HT_6 and 5-HT_7 [98]. In contrast to the 5-HT_1 to 5-HT_4 receptors, the 5-HT_5, 5-HT_6 and 5-HT_7 receptors have been less extensively investigated and much less is known about their functional properties.

As no selective ligands are available, little is known about the 5-HT_5 receptor [200]. 5-HT_5 receptors have not yet been demonstrated to 'function' in native systems [200]. It is likely that interest in 5-HT_5 pharmacology will follow once selective ligands are available.

Human 5-HT$_7$ receptors are positively coupled to an adenylate cyclase second messenger system. Several reasonably selective antagonists and agonists have been identified, and a number of leads for other structure types have been discovered. 5-HT$_7$ receptors have been implicated in a wide variety of pharmacological functions, although the functions and possible clinical relevance of this receptor are not yet fully understood. Indirect evidence suggests that 5-HT$_7$ receptor antagonism may be clinically useful in the treatment of depression and possibly involved in anxiety, epilepsy, pain, and schizophrenia [201]. Since the involvement of this receptor in schizophrenia is under investigation and no selective 5-HT7 antagonists have been developed for humans, this will not be further discussed in this introduction.

After rat and mouse 5-HT$_6$ receptors were described in 1993 and 1994 [202,203] and human 5-HT$_6$ receptors in 1996 [204], research in this area increased immensely. The human 5-HT$_6$ receptor is positively coupled to adenylate cyclase, has 89% overall sequence homology with the rat receptor and is nearly exclusively localized in the central nervous system [204]. It is predominantly found in the caudate nucleus, with lower concentrations in hippocampus and amygdala [204]. The first 5-HT$_6$-selective antagonist was described in 1998 [205]. Since then, various useful selective 5-HT$_6$ antagonists have been identified, some selective 5-HT$_6$ agonists have been reported and newer agents are developed with improved pharmacokinetic and pharmacodynamic properties [98,206].

Several recent lines of evidence have suggested a role of 5-HT$_6$ receptors in cognitive and memory processes, found in healthy animals and in animal models of cognitive impairment [98,109,207] and in patients with Alzheimer's disease (AD) [206,208]. Maher-Edwards *et al* performed an exploratory clinical study showing that the 5-HT$_6$ antagonist SB742457 and the acetylcholinesterase inhibitor donepezil improved global functioning of AD patients [209]. A combined 5-HT$_6$ receptor antagonist and cholinesterase inhibitor strategy could be a possible treatment of cognitive disorders such as those seen in Alzheimer's disease or schizophrenia. However, this class of drugs needs to be investigated more thoroughly and the role of this receptor in schizophrenia (even more than in AD) still needs to be defined.

The 5-HT$_6$ antagonist SB742457 is developed as a possible 'add-on' therapy for schizophrenia, to improve the negative symptoms or to decrease the cognitive side effects of currently used treatments. Since such compounds are designed to treat only part of the schizophrenic spectrum, they will usually be combined with other therapies. Therefore, interactions with other antipsychotic drugs are relevant, and in chapter 2 SB742457 was investigated in combination with risperidone. Risperidone is a D$_2$/5-HT$_{2A}$ antagonist with low affinity for 5-HT$_6$ receptors, which seems to produce a limited improvement on neurocognitive parameters in schizophrenic patients [210].

GLUTAMATERGIC STRATEGIES

Hypofunction of the glutamatergic system is one of the most promising non-dopaminergic theories for the pathophysiology of schizophrenia. Several targets have been explored to stimulate glutamateric activity [190,197], while avoiding potential adverse reactions like seizures or cell death [211]. Activation of metabotropic receptors indirectly induce ion channel formation and other postreceptor cascades [212]. The selective metabotropic glutamate 2/3 (mGlu2/3) receptor agonist LY404039 [213] has anxiolytic and antipsychotic effects in animal studies, without causing sedation [214] and is thought to work by reducing the presynaptic release of glutamate in brain regions where mGlu2/3 receptors are expressed [215,216]. In a first exploratory clinical study, it seemed effective in patients with schizophrenia and did not show major side effects [217].

Others have tried to stimulate glutamatergic activity through AMPA or kainate activation. AMPAkines, which potentiate AMPA transmission without binding directly to the agonist binding site [218], stimulate memory-dependent processing in animals [219] and could be useful to treat various aspects of schizophrenia. These compounds are currently under development for treatment of cognitive dysfunction in various neuropsychiatric disorders [119,220]. Although far less advanced, the AMPA/kainate receptor antagonist LY293558, showed effect in an animal model for cognitive deficits (ketamine), suggesting a possible utility of AMPA antagonists in the treatment of the cognitive deficits in schizophrenia [221].

The most developed way to augment NMDA receptor function indirectly is through facilitation of glycine, an obligatory co-agonist for glutamate at the NMDA receptor. As direct agonists of NMDA receptors are neurotoxic [114], indirect ways to enhance receptor function have been investigated [222]. After facilitation of glycine, an obligatory co-agonist for glutamate at the NMDA receptor, modest improvements in positive and negative symptoms and cognitive function in schizophrenic patients have been described in exploratory studies [55,211,211,223,224]. However, high doses of glycine are needed to significantly elevate CNS concentrations. Therefore, inhibition of presynaptic glycine reuptake seems a more efficient way to increase pharmacological glycine activity in the brain [225]. Of the two described glycine transporters (glycine transporter 1 or $GlyT_1$ and $GlyT_2$) [226], $GlyT_1$ seems to directly affect NMDA activity [226,227]. Glycine reuptake inhibitors have shown effects in several animal models of schizophrenia [228-230]. In a clinical trial, the glycine reuptake inhibitor sarcosine showed effects on positive and negative symptoms in schizophrenic patients [224,231]. As sarcosine is a low potency antagonist requiring gram-level dosing, more potent glycine reuptake inhibitors are now investigated, as is for example shown in chapters 4-7. In the study described in chapter 4, the first glycine reuptake inhibitor to be administered to humans was examined in healthy subjects. In the other two studies described in chapters 5 and 6, the glycine reuptake inhibitor, was administered to healthy men with and without scopolamine, to induce transient and reversible cognitive and memory impairments, and to reduce ceiling effects by prevention of performance at maximal capacity.

ENDOCANNABINOID STRATEGIES

As several lines of experimental and clinical evidence point to a dysregulation of the endocannabinoid system in schizophrenia, there is a potential for pharmacological manipulation of the endocannabinoid system as a novel approach for treating schizophrenia. Although experimental findings are still controversial, the CB receptor inverse agonist cannabidiol (CBD) and the CB_1 receptor antagonist rimonabant (SR141716) show the most consistent antipsychotic properties in dopamine- and glutamate-based (animal and human) models of schizophrenia, with profiles similar to atypical antipsychotic drugs [232].

CBD is one of the components of cannabis, which may constitute up to 40% of cannabis extracts and is devoid of the typical psychological effects of cannabis in humans [233]. The first evidence that CBD could have antipsychotic effects was obtained in an interactive study of CBD and deltag-THC in healthy volunteers published in 1982 [234]. The antipsychotic-like properties of CBD have been investigated in animal models using behavioural and neurochemical techniques, which suggested that CBD this compound may act as an atypical antipsychotic drug [233,235,236]. Also, in human models of psychotic symptoms in healthy volunteers, the antipsychotic-like activity of CBD was demonstrated [236]. Although CBD did not show effect in a study in treatment-resistant schizophrenic patients, a preliminary report from a 4-week clinical study suggested that CBD is an effective, safe and well-tolerated treatment for schizophrenic patients [236]. In 2007 Leweke *et al* found that CBD significantly reduced psychotic symptoms in acute schizophrenia with potency similar to amisulpride but with fewer side effects such as EPS, increase in prolactin, and weight gain [237].

Although the findings are variable, the selective CB_1 receptor antagonist rimonabant showed activity in preclinical models of antipsychotic efficacy. It failed, however, to demonstrate antipsychotic activity versus placebo and haloperidol in a clinical trial [238]. The ability of CBD and rimonabant to improve the psychosis-related cognitive impairment has not been sorted out yet and these compounds need further investigation for this indication [232].

Scope of this thesis

The introduction of this thesis gave an outline of some important pharmacological hypotheses of schizophrenia, the pharmacological properties of currently used antipsychotics, and compounds with new pharmacological mechanisms that are in the development pipeline.

This thesis describes how these pharmacological characteristics can be determined in healthy volunteers, at a stage of drug development when novel compounds are not yet tested in patients. The different chapters of this thesis illustrate how these concepts are used in practice, during the early development of new therapeutic strategies for schizophrenia. An important aim of these strategies is to identify treatments with an improved therapeutic window, since adverse events and therapeutic failures in schizophrenia form an important impediment in current medical practice.

One way to try to improve the therapeutic window of a drug therapy is by ameliorating the pharmacological mechanism of action, from direct, full and non-selective (D_2) receptor antagonists to more indirect, partial, or selective modulators. Chapters 2 and 4-6 carefully assessed the pharmacological characteristics of novel compounds, by using a large number of different 'drug biomarkers' to create a 'fingerprint' of the pharmacological effects. This is based on the notion that a drug's mechanism of action underlies both its desired and the undesired effects, and therefore provides an indication of the expected therapeutic window. Chapter 2 used a positive control to provide an indication of (dis) advantages of a potential antipsychotic drug from a new class (the NK_3 antagonist talnetant), compared to a widely used therapeutic congener (the D_2-receptor antagonist haloperidol).

Another way to improve the therapeutic window is by development of combination therapies, each targeted at a different clinical syndrome that characterize schizophrenia in addition to the well-known positive psychotic symptoms, in particular cognitive deficits (Chapter 3, examining a 5-HT6 antagonist). In each of these chapters, we used a multifunctional CNS test battery (the neuroCart) to make comprehensive pharmacological profiles of novel schizophrenia treatments in healthy subjects, which were compared with existing medications to identify potential pharmacological advantages of the new therapeutic strategies.

All studies were performed in healthy volunteers, which has the advantage that patients are not burdened with new drugs, at a stage when the therapeutic potential and adverse effect profile are still uncertain. The obvious disadvantage is that healthy volunteers do not have schizophrenia, making it impossible to demonstrate a therapeutic effect. Therefore, new human pharmacological models were developed that can be used in healthy volunteers to study aspects of the disease. Chapters 5 and 6 used a scopolamine model to induce reversible cholinergically mediated cognitive deficits in healthy volunteers, to examine how these 'negative symptoms' were affected by glycine reuptake inhibitors. Chapter 7 describes a pharmacokinetic/pharmacodynamic model of this scopolamine challenge test. In chapter 8, the cannabinoid Δ9-tetrahydrocannabinol (THC) was employed to temporarily cause a mild psychotic-like state in healthy subjects, and described how this was impacted by haloperidol. Thus, physiological perturbations in healthy subjects were used to mimic psychopathophysiological aspects of schizophrenia, in order to compare the effects of novel and established treatments at an early stage of drug development.

These approaches provide clear examples of how clinical pharmacological experiments in healthy volunteers can be used to characterize the pharmacological properties of novel compounds in development for schizophrenia, which basically underlie the therapeutic innovations that current drug development in this field tries to achieve. The studies also show that the main pharmacological focus in this area has shifted from psychosis to improvement of individual negative or cognitive symptom complexes, from direct receptor inhibition to indirect receptor modulation, and from single drug strategies to combination therapies. The diversity of drug development strategies in schizophrenia and related disorders reflects the increasing complexity of neuropharmacological hypotheses in this field. Despite these difficulties, incremental changes in drug characteristics and treatment strategies may well lead to the introduction of new classes or combinations of drugs in the future.

Figure 1 Dopamine pathways thought to be involved in the pathophysiology and adverse drug reactions of schizophrenia

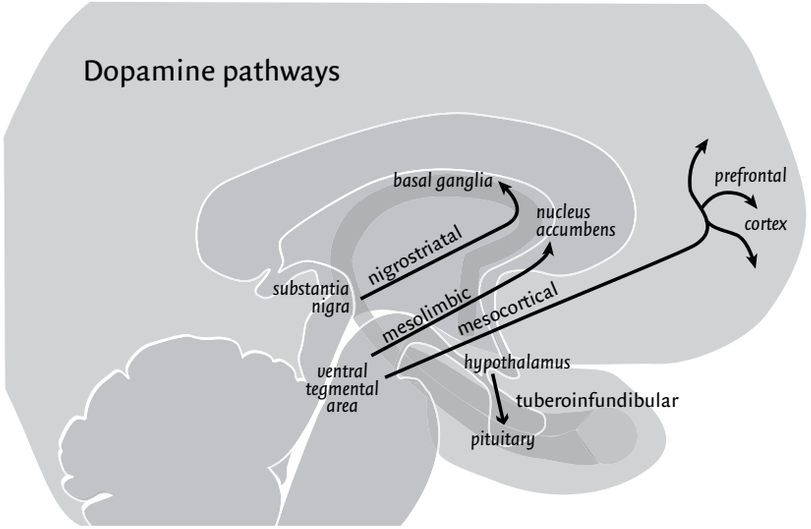


Figure 2 'Neuropharmacological combination model' of non-schizophrenic people (Glu=glutamate; $5HT_{2A}$=serotonin2A; DA=dopamine; GABA= gamma-aminobutyric acid)

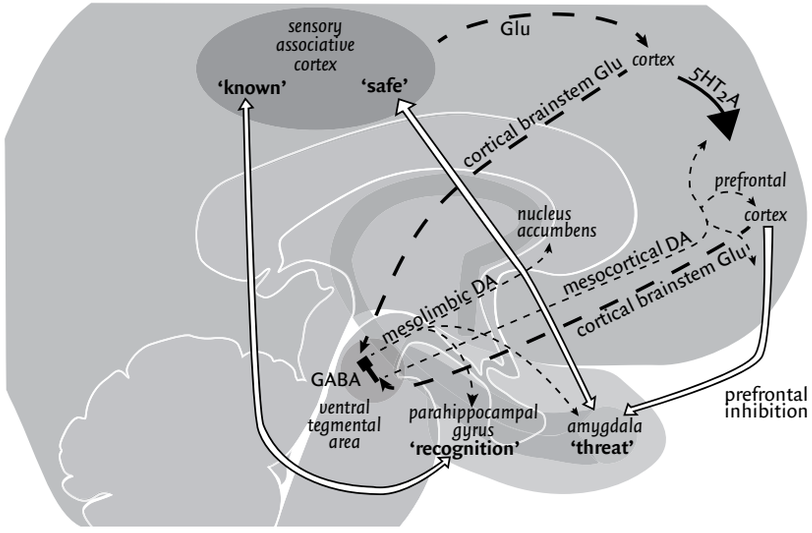
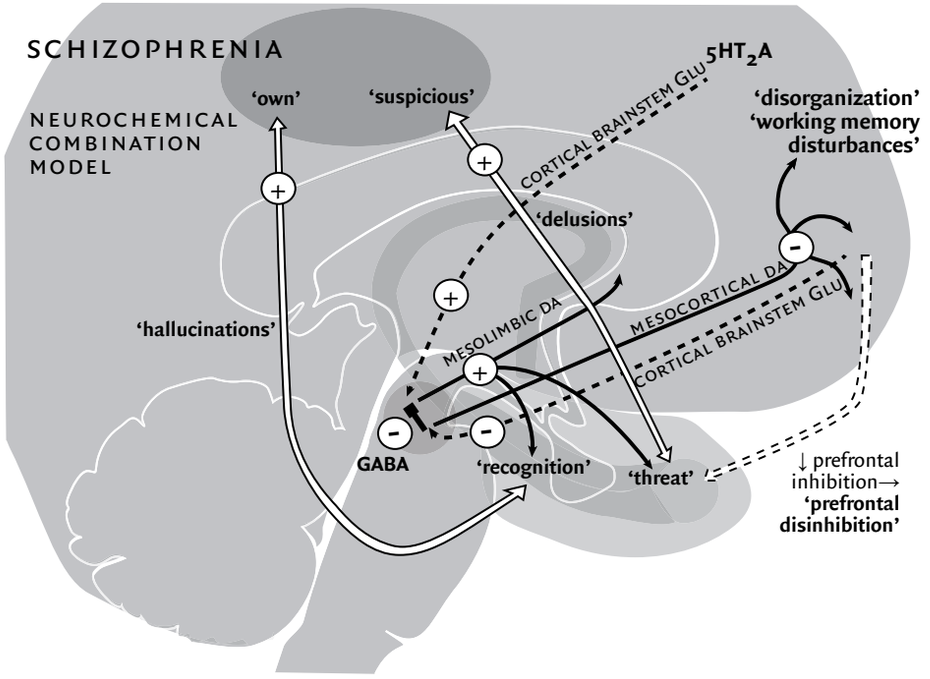


Figure 3 Visualized 'integrated neuropharmacological model' of schizophrenic patients (Glu=glutamate; $5HT_{2A}$=serotonin2A; DA=dopamine; GABA= gamma-aminobutyric acid)



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