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Modulation of prefrontal glutamatergic transmission and "atypicality" of antipsychotic drugs

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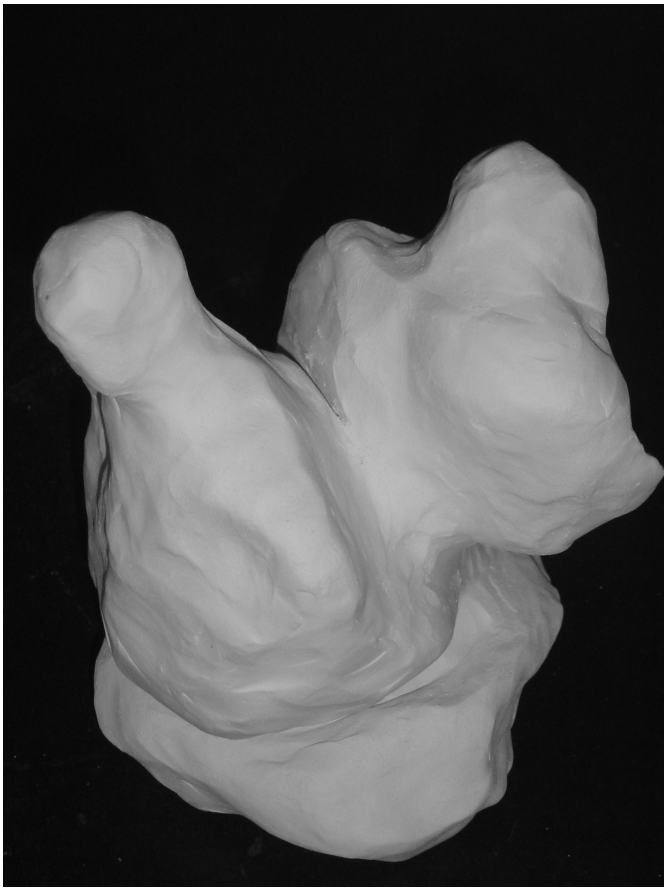

”Samma av två” sculpture by Mats Konradsson

ABSTRACT

The glutamate hypothesis of schizophrenia suggests a relationship between hypoactive NMDA receptor-mediated glutamatergic transmission and the pathophysiology of schizophrenia. Previous results have demonstrated that atypical (e.g. clozapine, risperidone, and olanzapine), but not typical (e.g. haloperidol, raclopride, and chlorpromazine) antipsychotic drugs, markedly potentiate NMDA receptor-mediated glutamateric transmission in the medial prefrontal cortex, an effect which was hypothesized to contribute to the ability of clozapine and other atypical antipsychotic drugs to ameliorate negative symptoms and cognitive dysfunctions in schizophrenia.

It has been suggested that intense tobacco consumption by schizophrenic patients may represent a form of self-medication with nicotine. Similarly to clozapine, nicotine has been proposed to improve both negative and cognitive symptoms in schizophrenic patients, and clozapine decreases smoking in these patients. By using electrophysiological techniques *in vitro* we have examined whether nicotine also facilitates NMDA receptor-mediated transmission in the rat medial prefrontal cortex, alone or in combination with the $D_{2/3}$ receptor antagonist raclopride or the weak D_4 receptor antagonist L-745,870. Neither nicotine nor raclopride or L-745,870 had any effect on NMDA-induced currents in pyramidal cells of the medial prefrontal cortex. However, the combination of nicotine with raclopride or L-745,870 produced a clozapine-like facilitation of the NMDA-induced currents in the medial prefrontal cortex. Similarly to clozapine, these drug combinations also potentiated electrically evoked excitatory potentials in the mPFC. These results suggest that the addition of nicotine to antagonists at D_2 and D_4 receptors may improve cognitive and negative symptoms in schizophrenic patients.

We have also investigated the effect of the glycine transporter-1 (GlyT-1) inhibitor NFPS on the NMDA-induced currents, both when given alone and in combination with either risperidone or clozapine. As reported previously, NFPS alone facilitated these currents. Moreover, NFPS augmented the effect of both a submaximal, and a maximal, concentration of risperidone on the NMDA-induced currents. In contrast, this GlyT-1 inhibitor did not potentiate either the effect of a submaximal or a maximal concentration of clozapine. These data may contribute to explain clinical studies suggesting that the efficacy of risperidone, but not clozapine, can be augmented by adjunctive treatment with agents acting directly or indirectly on the strychnine-insensitive glycine site on the NMDA receptor.

Several clinical studies have reported advantageous effects of using antiepileptic drugs as an adjunct to antipsychotic drugs in treatment-resistant schizophrenia. Topiramate, a new anticonvulsant drug is one of them. Previous studies suggest that topiramate may improve general psychopathologic and negative symptoms in schizophrenia when added to a stable medication. Topiramate also reverses weight gain in these patients, a common side effect of clozapine and some other antipsychotic drugs. In this thesis, we have also investigated topiramate on NMDA receptor-mediated glutamatergic transmission in the medial prefrontal cortex, both when given alone and in combination with raclopride or clozapine. Topiramate alone had no effect on the NMDA-induced currents. However, the combination of this anticonvulsant drug with raclopride generated a facilitating effect on these currents, which was blocked by the D_1 receptor antagonist SCH23390. Moreover, topiramate facilitated the effect of a submaximal, but inhibited the effect of a maximal concentration of clozapine. The combination of topiramate and raclopride also facilitated excitatory postsynaptic potentials in pyramidal cells of the medial prefrontal cortex. These data may contribute to explain clinical findings demonstrating that topiramate improves negative symptoms when added to typical antipsychotic drugs in the treatment of schizophrenic patients. These data may also have bearing on the deterioration of symptomatology seen with the combined topiramate and clozapine in schizophrenic patients.

Finally, we have investigated asenapine, a novel psychopharmacological agent which is developed for treatment of schizophrenia and bipolar disorder, by using a set of preclinical methods, which we previously used to explore antipsychotic drugs. Asenapine induced a dose-dependent suppression of conditioned avoidance response without any associated catalepsy. Our microdialysis studies showed that asenapine increased dopamine output in the medial prefrontal cortex, as well as in the striatum and the nucleus accumbens. A low dose asenapine increased dopamine efflux preferentially in the shell compared to the core of nucleus accumbens, as assessed by *in vivo* voltammetry. In similarity with clozapine, but at much lower concentrations, asenapine also facilitated the NMDA-induced currents in pyramidal cells of the medial prefrontal cortex. These results propose that asenapine may have a potent antipsychotic activity without generating extrapyramidal side effects, with effects not only on positive, but also on negative and cognitive symptoms.

LIST OF PUBLICATIONS

I. Jardemark K., Marcus M.M., Konradsson Å., Svensson T.H. The combination of nicotine with the D_2 antagonist raclopride or the weak D_4 antagonist L-745,870 generates a clozapine-like facilitation of NMDA receptor-mediated neurotransmission in pyramidal cells of the rat medial prefrontal cortex.
International Journal of Neuropsychopharmacology. 2005 Jun; 8(2):157-62.

II. Å. Konradsson, M.M. Marcus, P. Hertel, T.H. Svensson, K.E. Jardemark. Inhibition of the glycine transporter GlyT-1 potentiates the effect of risperidone, but not clozapine, on glutamatergic transmission in the rat medial prefrontal cortex.
Synapse. 2006 Aug; 60(2):102-8.

III. Å. Konradsson, M.M. Marcus, T.H. Svensson, K.E. Jardemark. Differential effects of topiramate on prefrontal glutamatergic transmission when combined with raclopride or clozapine.
Submitted to *International Journal of Neuropsychopharmacology.*

IV. O. Frånberg, C.Wiker, M.M. Marcus, Å. Konradsson, K. Jardemark, B. Schilström, M. Shahid, E.H.F. Wong, T.H. Svensson. Asenapine, a novel psychopharmacologic agent: preclinical evidence for clinical effects in schizophrenia.
Psychopharmacology (Berl) 2007 Oct; [*Epub ahead of print*].

CONTENTS

LIST OF ABBREVIATIONS

3-MT	3-methoxytyramine
5-HIAA	5-hydroxyindole acetic acid
5-HT	5-hydroxytryptamine, serotonin
AC	anterior cingulate
AGm	medial agranular
AMPA	α-Amino-3-hydroxy-5-methylisoxazole-4-propionic-acid
ANOVA	analysis of variance
AP	anterior posterior
CSF	cerebro spinal fluid
CNS	central nervous system
CNQX	6-cyano-7-nitroquinoxaline-2, 3-dione
COMT	catechol-O-methyl-transferase
CS	conditioned stimuli
DAT	dopamine transporter
DOPAC	dihydroxyphenylacetic acid
DL	dorsal lateral
EEA	excitatory amino acids
EPS	extrapyramidal side effects
EPSC	excitatory postsynaptic current
EPSP	excitatory postsynaptic potential
GABA	γ-aminobutyric acid
HPLC	high performance liquid chromotography
HVA	homovanillic acid
IL	infralimbic
i.p.	intraperitoneal
IP3	inositol triphosphate
i.v.	intravenous
L-Dopa	L-dihydroxyphenyl-alanine
LSD	lyseric acid diethylamide
mACh	muscarinic acetylcolinergic
MAO	monoamine oxidase
ML	medial lateral
MK-801	dizocilpine
NAc	nucleus accumbens
nACh	nicotinergic acethylcolinergic
MHPG	3-methoxy-4-hydroxy-phenylglycol
NMDA	*N*-methyl-D-aspartate
PET	positron emission tomography
PCP	phencyclidine, ”angel dust”
PL	prelimbic
PPI	prepuls inhibition
SN	substantia nigra
S.E.M.	standard error of mean
TTX	tetrodotoxin
UCS	unconditioned stimuli
VTA	ventral tegmental area

1 INTRODUCTION

1.1 Schizophrenia

Schizophrenia is a severe and disabling brain disorder with a world wide lifetime prevalence of approximately 1% in the general population, previously claimed to be independent of geographic, cultural or socio-economic variables (Sartorius *et al.*, 1986; Carpenter & Buchanan, 1994; Andreasen, 1995). However, more recent evidence proposes that poverty and low social class as well as upbringing in urban areas increases the risk of schizophrenia, as observed among immigrant populations in both Great Britain and the Netherlands (Jablensky, 1997). A high degree of heritability has also been shown in schizophrenia based on family studies (McGue & Gottesman, 1991), twin studies (Cannon *et al.*, 2000) and studies of adoptees (Kety *et al.*, 1976; Ingraham & Kety, 2000). The risk among first degree relatives is approximately 10-fold greater than in the general population (Harrison & Owen, 2003). Several putative susceptibility genes have been identified in schizophrenia and the evidence for some of them, such as for example DISC1, is relatively strong (Harrison & Weinberger, 2005; Ishizuka *et al.*, 2006; Porteous *et al.*, 2006; Ross *et al.*, 2006). Clearly, schizophrenia appears as a complex genetic disorder, and the products of a set of inherited genes may interact with each other and with environmental factors to induce the disease.

The onset of the disease, i.e. the patient's first psychotic episode, is often preceded by a prodromal phase, which include a number of non-specific symptoms or signs, such as e.g. depression and a general loss of interest, avoidance of social interactions as well as odd beliefs and behavior. The first psychotic episode usually occurs relatively early in life, and most patients need long-lasting medication, often during several decades.

Overall, schizophrenia manifests itself in a vast array of symptoms which fall into three broad categories, *positive*, *negative* and *cognitive* symptoms (Andreasen, 1995). *Positive* symptoms of schizophrenia reflect the presence of additional distinctly abnormal experience and behaviors related to delusions and hallucinations with marked bizarre or disorganised activity as a consequence. *Negative* symptoms reflect lack or decline in normal experience or behavior and include affective flattening, decreased thought and speech productivity, and these patients reflect the absence of normal interpersonal and social functions (social withdrawal), as well as decreased initiation of goal-oriented behavior. *Cognitive* symptoms include disorganised speech, thought disorder, disorganised behavior, deficits in learning and memory, lack of executive functions (e.g. abstract thinking and problem solving) and poor attention. Schizophrenia is also associated with a general lack of connection to reality, as well as emotional dysfunction. Individuals with schizophrenia may exhibit varying degrees of positive, negative and cognitive symptoms or a predominance of one cluster. The positive, negative and cognitive symptoms experienced by schizophrenic patients lead to great disability in their social life and work capacity, and a major deterioration of quality of life.

Schizophrenia is associated with mortality rates that are two to three times higher in those expected or observed in the general population (Brown, 1997; Auquier *et al.*, 2006).

The high mortality is due to the combination of a high risk of suicide (Nyman & Jonsson, 1986; Brown, 1997; Meltzer, 2002), and a heightened number of "natural deaths" (Auquier *et al.*, 2006). In 2000, Ösby and colleagues published a study on the mortality among all patients with a first hospital diagnosis of schizophrenia in Stockholm County, Sweden, during 1973-1995. A total of 7784 patients were included in the study, and it confirmed an increased mortality in schizophrenic patients. The largest single cause of death in both schizophrenic males and females was cardiovascular disease followed by suicide. The mortality caused by suicide or unspecified violence was particularly increased in patients of young age during the first year after the onset of the disease (Osby *et al.*, 2000).

1.2 The dopaminergic system

In the late 1950s, Carlsson and co-workers discovered that dopamine is a neurotransmitter in its own right within the central nervous system (CNS; Carlsson *et al*., 1957; Carlsson *et al.*, 1958). Furthermore, it was proposed that dopamine plays a key role in motor control by the basal ganglia, and that dopamine depletion in the striatum could be the cause of neurological symptoms in Parkinson's disease (Carlsson, 1959). We now know that the dopaminergic system is involved in and regulates also other behavioral functions, such as the emotional state, motivation, reward, attention and endocrine activity. Furthermore, the dopaminergic system has been implicated in several other brain disorders, such as schizophrenia, attention deficit disorder and drug dependence.

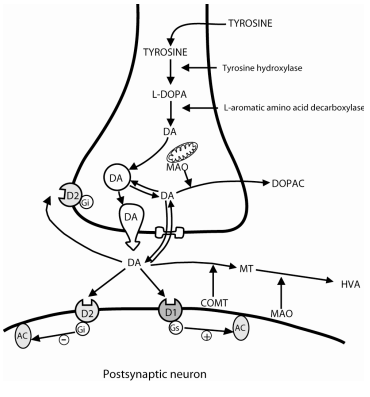

Figure 1. A schematic drawing of a dopaminergic nerve terminal. DA: dopamine; D1: D1-like receptor; D2: D2-like receptor. Other abbreviations see text. Modified from (Cooper *et al.,* 2003).

1.2.1 Biochemistry

Tyrosine, the precursor of the catecholamines, is derived from dietary sources and is transported into dopaminergic nerve terminals through a process of facilitated diffusion. Tyrosine is converted to L-dihydroxyphenyl-alanine (L-Dopa) by the enzyme tyrosine hydroxylase, an enzyme that is only found in catecholamine containing cells, and which is the rate limiting step in the synthesis of catecholamines (see Figure 1). L-dopa is decarboxylated to dopamine by L-aromatic amino acid decarboxylase. Dopamine is stored in presynaptic vesicles in the nerve terminal where it is protected from degradation. This process can be blocked by reserpine, which inhibits the storage of dopamine in the synaptic vesicles. The release of dopamine into the synaptic cleft is calcium dependent and triggered by an action potential which depolarizes the nerve terminal. The released dopamine is transported back into the nerv terminal by a dopamine transporter (DAT) and eliminated via conversion to dihydroxyphenylacetic acid (DOPAC) by intraneuronal monoamine oxidase (MAO).

Extracellular dopamine is converted to 3-methoxytyramine (3-MT) by catechol-O-methyl transferase (COMT). 3-MT is then further degraded to homovanillic acid (HVA) by MAO (Cooper *et al.*, 2003).

1.2.2 Dopamine pathways

The dopamine neurons are organized, into four different systems; *the nigrostriatal, the mesolimbic, the mesocortical and the tuberoinfundibular dopamine systems* (see Figure 2). The nigrostriatal dopamine system originates in the substantia nigra (SN) and projects primarily to the dorsal striatum. This system is involved in e.g. iniation of movements, habituation and sensorimotor coordination (Guillin *et al.*, 2007). The nigrostriatal dopamine system is involved in motor control as well as extrapyramidal side effects (EPS, see sction 1.9.2), which frequently may occur during treatment of schizophrenic patients with typical antipsychotic drugs. Degeneration of dopamine neurons in this pathway also causes symptoms of Parkinson's disease, e.g. inhibition of voluntary movements, muscular rigidity and tremor (see Hornykiewicz & Kish, 1987). Both the mesolimbic and mesocortical systems originate in the ventral tegmental area (VTA). The mesolimbic system projects to limbic structures such as ventral striatum, hippocampus, and amygdala, while the mesocortical system projects to cortical regions, e.g. to the prefrontal cortex. The mesolimbic and mesocortical systems are involved in regulation of emotional control, motivation, attention, reward, cognition and the reinforcing properties of many dependent producing drugs. Finally, the tuberoinfundibular system which is involved with endocrine control originates in the arcuate nucleus of the hypothalamus and projects to the pituitary stalk (Cooper *et al.*, 2003).

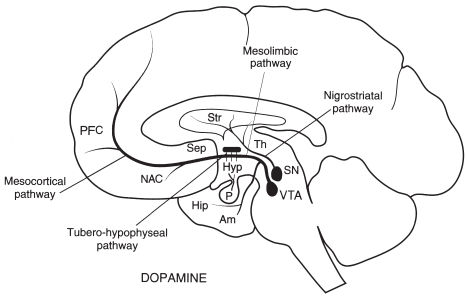

Figure 2. Dopamine pathways in the human brain.
Am: amygdaloid nucleus; Hipp: hippocampus;
Hyp: hypothalamus; NAC: nucleus accumbens; P: pituitary gland;
PFC: prefrontal cortex; Sep: septum; SN: substantia nigra;
Th: thalamus; VTA: ventral tegmental area.
Modified from (Rang *et al.*, 1999).

1.2.3 Dopamine receptors

There are currently five known dopamine receptor subtypes, termed D_1-D_5, and they are distinguished according to their sequential, functional and pharmacological characteristics (Spano *et al.*, 1978; Bunzow *et al.*, 1988). The dopamine receptors are divided into two main types, the D_1-family and the D_2-family, based upon their sequence homology. All of these receptors belong to the family of G-protein (G_s or G_i) coupled transmembrane receptors. The D_1-family, including the D_1 and D_5 receptors, are coupled to Gs, activates adenylyl cyclase, and increase cAMP formation, while the D_2-family, including the D_2, D_3, and D_4 receptors

are coupled to G_i, inhibit adenylyl cyclase, and decrease cAMP formation or increase inositol triphosphate (IP3; Kebabian & Calne, 1979; Kebabian *et al.*, 1984). Whereas all dopamine receptors are localized postsynaptically, the D_2 and D_3 receptors also function as presynaptic autoreceptors and blockade of D_2 receptors will result in an increase of dopamine synthesis and release, an effect that has also been attributed to a compensatory increase in firing rate of the dopamine neurones, induced by blockage of postsynaptic D_2 receptors (Bunney *et al.*, 1973; Cooper *et al.*, 2003). Dopamine autoreceptors are approximately tenfold more sensitive to dopamine and dopamine agonists than postsynaptic receptors (Roth, 1979).

The dopamine receptors differ in their regional localization in the rat brain. D_1 receptors show a widespread localization within brain regions receiving dopaminergic afferents, with highest densities in the striatum, nucleus accumbens (NAc), olfactory tubercle, and SN, but they are also found in thalamus, hypothalamus and cerebral cortex (Boyson *et al.*, 1986; Dubois *et al.*, 1986; Camps *et al.*, 1990). The D_1 receptors have been shown to play a major role in cognitive functions (Goldman-Rakic *et al.*, 2004). The D_5 receptors are mainly restricted to thalamic, hypothalamic and hippocampal areas (Meador-Woodruff *et al.*, 1992). D_2 receptors have been found in almost the same areas as D_1 receptors, however with highest densities in the striatum, olfactory tubercle, NAc, SN, and VTA. The D_2 receptors are also expressed in the pituitary gland where they regulate the secretion of prolactin. The D_3 receptors are mainly expressed in discrete brain areas within the limbic system (Sokoloff *et al.*, 1990), with the highest levels present in the medium sized spiny neurons of the rostral and ventromedal shell of NAc. These receptors are also expressed in the Islands of Calleja, and paleocerebellum with lower levels found in the ventral pallidum (Sokoloff *et al.*, 1990; Levesque *et al.*, 1992; Stanwood *et al.*, 2000). Moreover, D_3 mRNA is found in the SN pars compacta, and in the VTA, where it is expressed in a minority of the dopamine cells. D_4 receptors are predominantly found in cerebral cortex, but are less abundantly expressed in other brain regions (e.g. the basal ganglia) compared to other dopamine receptors. The D_4 receptors are localized on glutamatergic pyramidal neurons and γ-aminobutyric acid (GABA)ergic non-pyramidal neurons in both the cerebral cortex and hippocampus (Mrzljak *et al.*, 1996). It generally appears that D_2 receptors are predominantly expressed in areas associated with motor control while D_3 and D_4 receptors are preferentially located in areas associated with emotional and motivational proprties.

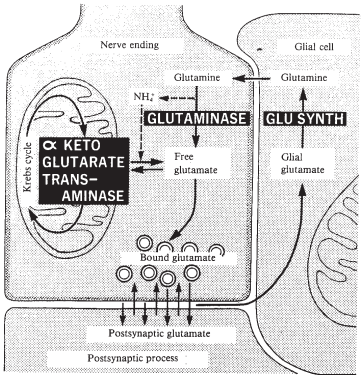

Figure 3. Glutamate synthesis. Adapted from Fundamentals of Neuropsychopharmacology. (Leonard, 1987).

1.3 The Glutamatergic system

Glutamate is the major excitatory neurotransmitter in the mammalian brain, accounting for roughly 60 % of the neurons and is utilized by 40% of all synapses. This amino acid is believed to modulate and regulate sensory, motor, cognitive and emotional functions and is also a critical component in the generation in synaptic plasticity, i.e. long-lasting changes in synaptic function or efficacy. Synaptic plasticity is

believed to underlie some forms of learning and memory. Glutamate is the specific neurotransmitter of the pyramidal cells in the prefrontal cortex.

1.3.1 Biochemistry

Glutamate is synthesized in the nerve terminals from either glucose, via the Krebs cycle, or from glutamine (see Figure 3). Glutamine is transported across the blood-brain barrier with high affinity and is available at high extracellular concentrations in brain and cerebro spinal fluid. Glutamine is also synthesized in glial cells, transported into nerve terminals and converted to glutamate by glutaminase (Scatton, 1993; Cooper *et al.*, 2003). Following Ca^{2+} dependent release, glutamate is, *via* an energy-dependent transport process, rapidly removed from the synaptic cleft both *via* neuronal and glial glutamate transporters. Glutamate in glial cells is converted to glutamine by glutamine synthetase (Cooper *et al.*, 2003).

1.3.2 Glutamatergic pathways

In the mammalian brain most of the glutamate pathways originate in the cerebral cortex and innervate most of the subcortical regions as well as the spinal cord. Functionally important glutamatergic pathways are the corticostriatal pathway, the perforant pathway (entorhinal cortex→hippocampus) and the climbing fibres (inferior olive→Purkinje cells) Most of the cortico-cortical connections as well as primary afferent terminals to the spinal cord also contain glutamatergic fibres (Scatton, 1993).

1.3.3 Glutamate receptors

The glutamate receptors are divided into ligand-gated ionotrophic and metabotropic glutamate receptors. The ionotrophic glutamate receptors, which are receptor/ion-channel complexes, can be subdivided into three subtypes (see Figure 4): the α-Amino-3-hydroxy-5-methyl-isoxazole-4-propionic-acid (AMPA) receptors, the kainate receptors and the *N*-methyl-D-aspartate (NMDA) receptors. The metabotrophic (G-protein coupled) glutamate receptors modify cellular functions via activation or inhibition of intracellular cascade processes.

Ionotrophic glutamate receptors

- **NMDA**
  widely distributed in mammalian CNS (enriched in hippocampus and cerebral cortex)
- **AMPA**
  widespread in CNS; parallel distribution to NMDA receptors
- **Kainate**
  concentrated in a few specific areas of CNS, complementary to NMDA/AMPA distribution

(Cooper *et al.*, 2003)

1.3.3.1 The NMDA receptor

Autoradiographic studies have shown that NMDA receptors are widely distributed in the rat brain (Buller *et al.*, 1994). The NMDA receptors have critical roles in excitatory synaptic transmission as well as in learning and memory functions, plasticity (O'Brien *et al.*, 1998; Dingledine *et al.*, 1999) and excitotoxicity in the CNS. The involvement of NMDA receptors in these diverse processes reflects their unique features, i.e. *i*) they are blocked by extracellular Mg^{2+} in a voltage dependent manner, *ii*) they have a high permeability to Ca^{2+}, Na^{+} and K^{+} and, *iii*) they have slow activation/deactivation kinetics. NMDA receptors also display sensitivity to an array of endogenous ligands and modulators. The co-agonists glycine, D-serine or D-alanine are essential for the activation of the NMDA receptor, whereas physiological levels of protons suppress activation of this receptor. Extracellular Zn^{2+} produces a voltage-independent block and polyamines (e.g. spermine and spermidine)

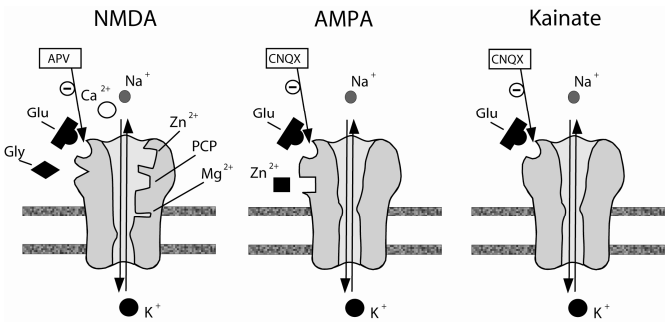

Figure 4. Schematic drawing of the ionic excitatory receptors. APV: 2-amino-5-phosphono-valeric acid; CNQX: 6-cyano-7-nitroquinoxaline; Glu: glutamate; Gly: glycine; PCP: phencyclidine. Modified from (Kandel *et al.*, 1991).

function as allosteric modulators of NMDA receptors, but only in the presence of glutamate and glycine.

The NMDA receptor consists the subunits, NR1, NR2 (A, B, C, D) and NR3 (A and B) (Goebel & Poosch, 1999; Cull-Candy *et al.*, 2001) and all NMDA receptors appear to function as heteromeric assemblies composed of multiple NR1 subunits in combinations with at least one type of NR2. The NR3 subunits do not form functional receptors alone, but can co-assemble with NR1/NR2 complexes (Perez-Otano *et al.*, 2001). Different properties of the NMDA receptor depend on the subunit composition. For example, the NR1 subunit is essential for the activation of the receptor, including agonist and antagonist selectivity, co-agonist modulation, polyamine activation, voltage-dependent Mg^{2+} block, Ca^{2+} permeability and Zn^{2+} inhibition (Nakanishi, 1992). Moreover, NMDA receptor subunits interact with various intercellular scaffolding, anchoring and signalling proteins and molecules associated with the postsynaptic density (Cull-Candy *et al.*, 2001). A distinctive feature of the NMDA receptor is that it is relatively inactivated under normal physiological conditions (Scatton, 1993; Cooper *et al.*, 2003). Over-activation or prolonged stimulation of the NMDA receptor, e.g. by loss of Mg^{2+} block and/or increased synthesis of polyamines and glycine (D-serine or D-alanine), has been linked to epileptic discharges and to neuronal cell death following cerebral ischemia and trauma.

NMDA receptors are blocked in a non-competitive way by phencyclidine (PCP), ketamine and dizocilpine (MK-801), which all bind to the PCP-site within the ion channel of the receptor. The ability of PCP to induce schizophrenia-like symptoms represents one of the strongest pieces of evidence linking glutamatergic dysfunction to the pathophysiology of schizophrenia (see below, section 1.4.2.1).

1.3.3.2 Non- NMDA receptors

Both AMPA and kainate receptors are, in general, permeable to Na^+ and K^+, and they mediate, in contrast to, the NMDA receptors fast excitatory synaptic transmission (Cooper *et al.*, 2003). AMPA receptors are widely expressed in the brain, co-localized with NMDA receptors at individual excitatory synapses and are composed of combinations of GluR1-4 subunits. The AMPA receptors provide the primary depolarization of the cell which is needed for release of the Mg^{2+} block of the NMDA receptors, thereby permitting Ca^{2+} influx in the cell. Activity of AMPA receptors are thought to be responsible for the expression of synaptic plasticity, whereas NMDA receptors are responsible for its control (i.e. Ca^{2+} influx), that triggers the active insertion or removal of AMPA receptors. The kainate receptors are composed of GluR5-7 subunits. Kainate receptors are expressed in cells in a few specific areas of CNS, complementary to the NMDA/AMPA receptors distribution (Javitt, 2007).The functional role *in vivo* of kainate receptors is uncertain (Sprengel & Seeburg, 1993) but theyare believed to be involved in synaptic plasticity (Rang *et al.*, 2007).

1.3.3.3 Metabotropic glutamate receptors

Metabotropic glutamate receptors provide regulation of presynaptic glutamate release and postsynaptic sensitivity. They execute their effects on ion channels via protein phosphorylation and second messenger systems (Cooper *et al.*, 2003). The metabotropic receptors are divided into three groups based on their functional activity, sequence similarities, signal-transduction properties, and pharmacological profile. Group I ($mGluR_1$ and $mGluR_5$) receptors stimulate IP3 hydrolysis/Ca^{2+} signal transduction. Group II ($mGluR_2$ and $mGluR_3$) receptors inhibit adenylyl cyclase and decrease cAMP. Finally, group III ($mGluR_4$, $mGluR_6$, $mGluR_7$ and $mGluR_8$) receptors also negatively linked to adenylyl cyclase activity. (Cooper *et al.*, 2003). The metabotropic glutamate receptors are widely expressed throughout the brain, but the different groups show some differential distribution.

1.4 Hypotheses of schizophrenia and dopamine-glutamate interaction

1.4.1 The classical dopamine hypothesis

The original hypothesis concerning the pathophysiology of schizophrenia proposed an overall hyperactivity of dopamine in brain, since all drugs that possessed antipsychotic efficacy found to bind and block D_2 receptors, and thus to impair dopaminergic activity in brain. In 1963, Carlsson and Lindqvist reported the first evidence supporting this hypothesis. They observed that the antipsychotic drugs chlorpromazine and haloperidol shared a common

ability to enhance the metabolism of dopamine and noradrenaline, an effect which was interpreted as a compensatory mechanism due to blockade of postsynaptic catecholamine receptors (Carlsson & Lindqvist, 1963). In support of this hypothesis, several studies using receptor ligand binding techniques *in vitro* subsequently showed that all clinical effective antipsychotic drugs bind to D_2 receptors in brain (Seeman *et al.*, 1975; Creese *et al.*, 1976; Creese *et al.*, 1977). By using positron emission tomography (PET) antipsychotic drugs were later shown to label dopamine receptors in the brains of schizophrenic patients (see Farde *et al.*, 1988; Farde & Nordstrom, 1992; Farde *et al.*, 1992; see section antipsychotic drugs 1.9). Moreover, Nordström and co-workers observed a statistically significant relationship between antipsychotic effect and D_2 occupancy in schizophrenic patients (Nordstrom *et al.*, 1993). Other evidence supporting a dopaminergic hyperactivity in the brain in these patients is provided by the finding that dopamine agonists as well as drugs such as amphetamine, which stimulates dopamine release, usually worsens psychosis in patients with schizophrenia (Snyder *et al.*, 1974). These drugs may also, in healthy volunteers, induce paranoid psychosis and symptoms that generally resemble the positive symptoms of schizophrenia (Angrist *et al.*, 1974). Recent observations using brain imaging techniques have shown evidence for an increased striatal dopamine release and metabolism in schizophrenic patients (Reith *et al.*, 1994; Laruelle *et al.*, 1996; Breier *et al.*, 1997; Lindstrom *et al.*, 1999).

1.4.2 The glutamate hypothesis of schizophrenia

1.4.2.1 The glutamate hypofunction hypothesis of schizophrenia

The glutamate deficiency hypothesis proposes a relationship between an impaired glutamatergic neurotransmission, particularly at the NMDA receptor level, and the pathophysiology of schizophrenia. The hypothesis is based on several findings: *i*) PCP (see section 1.3.3.1), was found to cause hallucinations and delusions as well as formal thought disorder even in healthy volunteers, symptoms that are distinctive features of schizophrenia (Luby *et al.*, 1959; Luby *et al.*, 1962), *ii*) in schizophrenics, NMDA receptor antagonists can produce an exacerbation of psychotic symptoms and cognitive impairments (Luby *et al.*, 1959; Javitt & Zukin, 1991), *iii*) PCP- or ketamine- treated rats have been observed to display cognitive deficits (Danysz *et al.*, 1988; Alessandri *et al.*, 1989; Verma & Moghaddam, 1996), *iv*) the non-competitive NMDA receptor antagonist ketamine was found to generate impairment in the manipulation of information within working memory in healthy volunteers (Honey *et al.*, 2003), *v*) clinical studies show that compounds (e.g. D-serine, glycine and D-cycloserine) acting on the glycine site of the NMDA receptor may reduce negative symptoms in schizophrenic patients treated with typical (Heresco-Levy *et al.*, 1996; Goff *et al.*, 1999b) and some atypical antipsychotic drugs e.g. risperidone (Evins *et al.*, 2002; Heresco-Levy *et al.*, 2004) and olanzapine (Heresco-Levy *et al.*, 2005), *vi*) it has been shown that several of the genes that are linked to the susceptibility to develop schizophrenia express proteins that are involved in modulating the function of NMDA receptors (Harrison & Owen, 2003; see Moghaddam, 2003), *vii*) several research groups have observed an altered expression of NMDA receptor subtypes in the prefrontal cortex of schizophrenic patients (see e.g. (Akbarian *et al.*, 1996; Kristiansen *et al.*, 2007). Finally, mice expressing only 5% of normal

levels of one of the essential NMDA receptor subunits (NR1), display schizophrenia-like behavioral abnormalities (Mohn *et al.*, 1999).

1.4.2.2 The role of kynurenic acid

A recent hypothesis related to the glutamate hypofunctioning hypothesis is the kynurenic acid hypothesis, based on the observation that kynurenic acid (see Figure 5), an endogenous NMDA and α7 nicotinic acetylcholine (nACh) receptor antagonist (Hilmas *et al.*, 2001), is elevated in the CSF (Erhardt *et al.*, 2001) and brain from schizophrenic patients (Schwarcz *et al.*, 2001). Furthermore, mRNAs for enzymes involved in the biosynthesis of kynurenic acid have been found elevated in schizophrenic patients (Miller *et al.*, 2004). Several preclinical studies lend further support to this hypothesis. In similarity with other NMDA antagonists (e.g. PCP, MK-801 and ketamine), acute administration of kynurenic acid disrupts prepulse inhibition (PPI; Erhardt *et al.*, 2004), a method used to quantify complex sensorimotor gating processes in the brain. PPI has also been found to be disrupted in schizophrenic patients. Moreover, kynurenic acid has been shown to reduce extracellular glutamate release in the striatum of the rat *in vivo* (Carpenedo *et al.*, 2001), an effect thought to be mediated by a α_7 nACh receptor dependent mechanism.

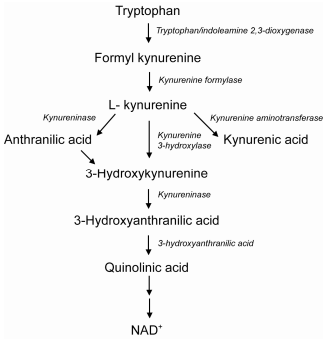

Figure 5. The kynurenine pathway (Nilsson, 2005; Erhardt *et al.*, 2007).

1.4.3 Hypofrontality and the modified dopamine hypothesis in schizophrenia

The prefrontal cortex has been implicated in a variety of cognitive functions, e.g. planning, abstract reasoning, problem solving, social interactions, and working memory. In 1974, Ingvar and Franzén coined the term hypofrontality based on their finding that the ratio of frontal to post-central blood flow is decreased in schizophrenic patients (Ingvar & Franzen, 1974; Franzen & Ingvar, 1975). Since then hypofrontality in schizophrenia has been studied by a number of methods, including measurements of blood flow in animal models of working memory, and multiple techniques of cognitive psychology and neuropsychology. Although, these studies support a prefrontal dysfunction in schizophrenia (see e.g. Weinberger *et al.*, 1986; Andreasen *et al.*, 1992; Yurgelun-Todd *et al.*, 1996), the findings are not entirely consistent, and some researchers have even observed an increased activation of the prefrontal cortex in schizophrenic patients during working memory tests (Manoach *et al.*, 1999; Callicott *et al.*, 2000). Post-mortem studies have shown atrophy of neurons and loss of neuronal prosesses and synapses in cortex of schizophrenic patients (Goldman-Rakic & Selemon, 1997). Thus, the majority of clinical and preclinical studies support a prefrontal dysfunction in these patients, and this dysfunction has also been found to be correlated with negative and cognitive symptoms of schizophrenia (Franzen & Ingvar, 1974; Ingvar & Franzen, 1974; Knable & Weinberger, 1997).

A dysfunctioning prefrontal cortex in schizophrenic patients may also involve a dysfunction of prefrontal glutamatergic transmission. The firing activity of VTA dopamine neurons, in particular their burst firing mode, has been shown to be driven by glutamatergic afferents impinging upon the dendrites of midbrain dopamine neurons (Chergui *et al.*, 1993; Murase *et al.*, 1993a), and burst firing evokes larger release of dopamine in target areas than regular firing (Gonon, 1988). Subsequent studies showed that the burst firing mode is also associated with much more prominent effects in dopamine target areas, as reflected by changes in immediate early gene expression (Chergui *et al.*, 1996; Chergui *et al.*, 1997). Indeed, several studies have shown that functional inactivation of the prefrontal cortex specifically reduces the burst firing of VTA neurons (Svensson & Tung, 1989; Charlety *et al.*, 1991; Murase *et al.*, 1993a). Interestingly, preclinical data revealed that whereas low doses of systemic MK-801 specifically reduced burst firing in dopamine neurons projecting to the prefrontal cortex of the rat, the same treatment induced a high frequency, monotonous firing in dopamine neurons projecting to subcortical areas (Murase *et al.*, 1993b). Thus, dysfunction of the prefrontal cortex may generally be related to impaired prefrontal dopaminergic functioning, since the VTA neurons that project to the prefrontal cortex are the same as those that receive a direct glutamatergic input from the prefrontal cortex. In addition, a dysfunctional prefrontal dopaminergic transmission might be secondary to a downregulation of cortical NMDA receptor function. Neuroimaging studies in rodents and primates show that administration of PCP results in decreased dopamine levels and a upregulated binding of the D_1 receptor ligand [^{11}C]NNC112 in the prefrontal cortex (Jentsch *et al.*, 1997a; Jentsch *et al.*, 1997b; Tsukada *et al.*, 2005), which may represent a compensatory response to downregulated prefrontal dopamine transmission. In fact, an increased D_1 ligand bindning in the dorsolateral prefrontal cortex has been observed in patients with schizophrenia (Abi-Dargham *et al.*, 2002). A hypofunction of prefrontal dopaminergic transmission may also be related to an amino acid polymorphism (VAL158MET) in the COMT gene, which affects the activity level of COMT, and hence the catabolism of dopamine. This polymorphism has been found to predict performance on dopamine-dependent prefrontal tasks in healthy adults and patients with schizophrenia (Egan *et al.*, 2001). Consequently, both hypo- and hyperfunctioning dopaminergic transmission may be involved in schizophrenia. The modified dopamine hypothesis thus proposes an increased dopamine release and stimulation of subcortical D_2 receptors, which is associated with psychiatric symptoms, concomitant with a hypofunctioning prefrontal dopamine transmission, which is associated with negative symptoms and cognitive deficits in schizophrenia (Svensson, 2000). Moreover, Carlsson *et al* (Carlsson *et al.*, 2001) have proposed that dysfunctions of both dopaminergic and glutamatergic transmission may affect striatal GABA-ergic neurons, which may exert an inhibitory action on thalamocortical

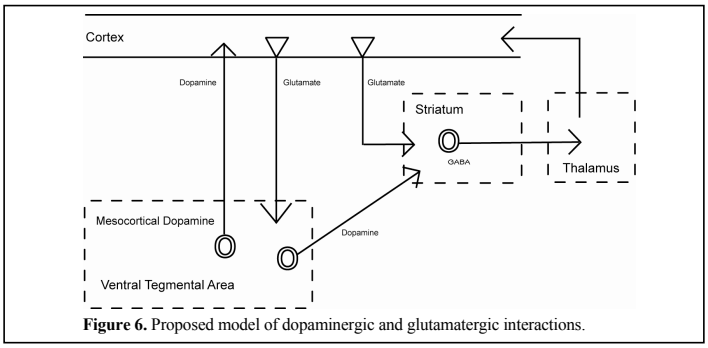

Figure 6. Proposed model of dopaminergic and glutamatergic interactions.

neurons (see Figure 6). Thalamus is thought to act as a filter for sensory information, and hyperactivity of dopamine or hypofunction of corticalstriatal glutamate transmission should impair this filter which may generate psychosis.

1.5 The Noradrenergic system

In the middle of the 1950s noradrenaline was shown to function as a neurotransmitter in the central nervous system (Vogt, 1954). Early studies in awake animals, in particularly the monkey, revealed that activity of the locus coeruleus (LC) noradrenergic system is correlated with vigilance, showing phasic activation responses to environmental sensory stimuli, particularly if associated with novelty of fear, but showing low activity in association with behaviors such as grooming, sweet water consumption or sleep (Foote *et al.*, 1983). Thus, a role in attentional functioning was confirmed and, in principle, the system may serve as a significance enhancer with respect to salient environmental stimuli. As mentioned above, this function also appears to apply to the internal milieu, and a general role in the so-called defence reaction appears very likely (Svensson, 1987). Subsequent studies over the past two decades have largely confirmed and extended such a biological role of the largest brain noradrenergic system (Aston-Jones *et al.*, 1999) and, accordingly, it is not suprising that brain noradrenaline systems have been found to be involved in the mode of action of both antidepressant drugs (see Svensson, 2000; Harro & Oreland, 2001) as well as a number of antipsychotics (see below), and drugs affecting wakefulness and attention, such as nicotine (Svensson & Engberg, 1980; Mitchell, 1993).

1.5.1 Biochemistry

The synthetic pathway of noradrenaline is similar to that of dopamine (see Figure 7). The original precursor is tyrosine and in noradrenergic neurons dopamine is further converted to noradrenaline by the enzyme dopamine β-hydroxylase, located in the synaptic vesicles. High concentrations of synthesized noradrenaline are stored in the vesicles together with ATP, and the content of these vesicles is released via an exocytosis process (Rang *et al.*, 2007). Extracellular noradrenaline is metabolized to normetanephrine by COMT and then to 3-methoxy-4-hydroxyphenylglycol by MAO. The released noradrenaline is largely transported back into the nerve terminal by the noradrenaline transporter and then degraded by intracellular MAO to 3-methoxy-4-hydroxy-phenylglycol (MHPG).

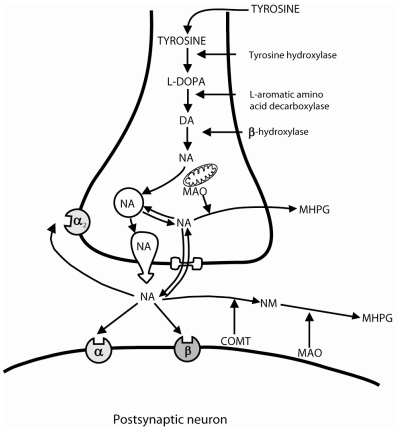

Figure 7. Schematic drawing of a noradrenergic neuron. α: α adrenoceptors, β: β adrenoceptors, NA: noradrenaline, NM: normetanephrine; MHPG: 3-methoxy-4-hydroxy-phenylglycol. Modified from (Cooper *et al.*, 2003).

1.5.2 Noradrenergic pathways

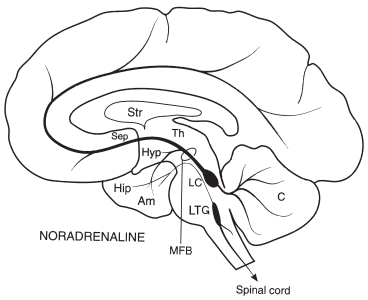

Figure 8. Noradrenergic pathways in the human brain. Am: amygdaloid nucleus; C: cerebellum; Hip: hippocampus; Hyp: hypothalamus; LC: locus coeruleus; LTG: lateral tegmental group; MFB: medial forebrain bundle; Sep: septum; Str: striatum; Th; thalamus. Modified from (Rang *et al.*, 1999).

The noradrenergic cell bodies are localized in the brain stem and can be divided into two major subgroups, the LC nucleus and the lateral tegmental nuclei (see Figure 8). The LC is located in the preventricular gray matter and contains approximately 15000 neurons on each side of the pons in the rat (Swanson, 1976). The axons of these neurons form extensive collateral branches with wide projections to the brain. The LC projects to e.g. the cerebellum, hippocampus, amygdala and most cortical areas (Dahlström & Fuxe, 1964), as well as to the brain stem and the spinal cord. The lateral tegmental nuclei has its cells of origin distributed diffusely in the medulla and pons and innervates primarily the brainstem, the hypothalamus and parts of the amygdala, and these cells also send descending projections to the spinal cord.

1.5.3 Noradrenaline receptors

The adrenoceptors all belong to the G-protein coupled superfamily and are localized both pre-and postsynaptically. They can also be expressed in glial cells (see Svensson & Mathe, 2002). The classification into α and β adrenergic receptors was originally based on the response of sympathetically innervated peripheral organs and the use of antagonists to these receptors. The adrenoceptors have been divided into α_1 (A, B and D) and α_2 (B, C and D) and β_1 and β_2 (Bylund, 1988; Docherty, 1998). The α_1-receptors are excitatory and activate phospholipase C, which in turn produces the second messenger IP3. In contrast, the α_2-receptors hyperpolarize the cell via inhibition of adenylyl cyclase and decreasing cAMP. All types of β-receptors stimulate adenylyl cyclase and thus increase the formation of cAMP. Both α_{1A} and α_{1B} receptors, respectively, are widely expressed in brain and located postsynaptically. The α_2-receptors were originally considered to be presynaptic (Langer, 1974) and high α_2 receptor densities were found in the LC, the amygdala, the hippocampus and the prefrontal cortex. More recent data show that α_2 receptors also can be postsynaptically located and that α_1 receptors also can be presynaptically located (Docherty, 1998). The β_1-receptors predominate in the cerebral cortex, the dendate gyrus, and medial dorsal nuclei, whereas the β_2-receptors are more abundant in the cerebellum, the paraventricular and the central thalamic nuclei.

1.5.4 Noradrenaline and schizophrenia

Clinical studies report significant increases in both cerebro spinal fluid (CSF) and plasma levels of noradrenaline in schizophrenic patients compared to controls. These findings

support a link between central noradrenergic hyperactivity and enhanced arousal in schizophrenic patient (Kemali *et al.*, 1982). Moreover, blockage of both α_1 and α_2 adrenoceptors have been suggested to be involved in the mechanisms of action of antipsychotic drugs (see Svensson, 2003).

1.6 The Cholinergic system

Brain cholinergic systems are believed to play an essential role in learning and memory (Bartus *et al.*, 1982), as well as to modulate sensory, motor and arousal tone (Mesulam *et al.*, 1983). Diseases such as schizophrenia, Alzheimer's disease and dementia are thought to be associated with dysfunctional cholinergic transmission (Davies & Maloney, 1976).

1.6.1 Biochemistry

Acetylcholine is synthesized from choline, which is transported back into the nerve terminal by a specific carrier (see Figure 9). Similarily to dopamine, noradrenaline and glutamate, newly synthesized acetylcholine is stored in vesicles and released into the synaptic cleft by a Ca^{2+} dependent exocytosis. Extracellular acetylcholine interacts with receptors or is rapidly inactivated to choline and acetic acid by the enzyme acetylcholine esterase.

1.6.2 Cholinergic pathways

The cholinergic neurons in the rat brain are divided into six groups (Ch1-Ch6), which give rise to the forebrain cholinergic system (Ch1-Ch4) and the brainstem cholinergic systems (Ch5-6). The neurons of the Ch1 group originate in the septum and the Ch2 neurons in the vertical limb of the diagonal band, and these neurons provide the major cholinergic projection to the hippocampus. The Ch3 group is most limited to the lateral part of the horizontal limb nucleus and project to the olfactory bulb. Ch4 neurons projects to the neocortex and amygdala and originate mainly in the nuclus basalis magnocellularis. In the brainstem, neurons of the Ch5 originate in the nucleus pedunculopontine, while the Ch 6 neurons originates in the laterodorsal tegmental nucleus, and their projections provide the cholinergic input of thalamus (Mesulam *et al.*, 1983)

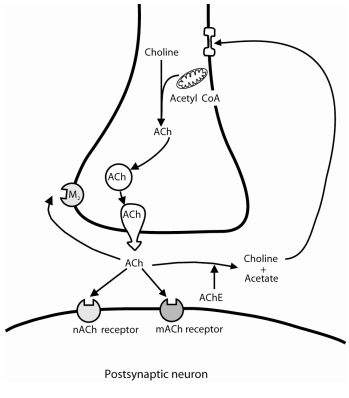

Figure 9. Schematic drawing of a cholinergic nerve terminal. AChE: acetylcholine esterase; M_2: muscarinic M_2 receptor; mAChR: muscarinic acetylcholine receptor; nAChR: nicotinic acetylcholine receptor. Modified from (Cooper *et al.*, 2003).

1.6.3 Cholinergic receptors

Cholinergic receptors are divided into two classes, muscarinic (mACh) and nACh receptors. Three main types of mACh receptors have been cloned, and are named M_1-M_5. The muscarinic receptors are found throughout the brain, including cortex, striatum, hippocampus, thalamus and the brainstem. All of the mACh receptors are G-protein coupled receptors and act directly on ion channels or are linked to different second-messenger systems. The M_1, M_3 and M_5 are coupled to IP3, while M_2 and M_4 are coupled to cAMP. The activation of these receptors has been shown to modulate voltage dependent ion-channels, K^+-channels, Cl^--channels and Ca^{2+}- channels. Thus, stimulation of muscarinic receptors can both depolarize and hyperpolarize the cell. The nACh receptors are ligand-gated ion channels permeable to Na^+, K^+ or Ca^{2+}, and they mediate fast excitatory synaptic transmission. nACh receptors are expressed in the autonomic ganglia, neuromuscular junction, and throughout the brain. Neuronal nACh receptors are pentamers and contain only subunits from the subgroups, α and β (α2-10 and β2-4). Notably, the exact composition of naturally occurring nACh receptors in brain is diverse. The nACh receptor-mediated cholinergic transmission regulates transmitter release, cellular excitability and neuronal integration, which are crucial for network operations and psychological processes, such as cognitive functions. For example, mechanistic studies have shown that the α_7 and $\alpha_4\beta_2$ nACh receptors in the hippocampus are critical for the cholinergic involvement in cognitive functioning (Rezvani & Levin, 2001; Levin & Rezvani, 2007).

1.6.4 The cholinergic system and schizophrenia

Altered cholinergic function in the brain has been suggested as a contributing factor to the pathophysiology of schizophrenia. For example, in post-mortem studies, it has been shown that schizophrenic patients have a reduced number of nACh receptors in different brain regions, e.g. hippocampus (Freedman *et al.*, 1995; Rezvani & Levin, 2001), thalamus and frontal cortex (Martin-Ruiz *et al.*, 2003). Furthermore, an altered expression and function of the α_7 nACh receptor have been suggested to contribute to the pathophysiology of schizophrenia (Leonard *et al.*, 1996; Adler *et al.*, 1998; Freedman *et al.*, 2000).

It is well established that schizophrenic patients smoke considerally more than healthy subjects and even other mentally ill patients (Hughes *et al.*, 1986). Thus, the prevalence of cigarette smoking among patients with schizophrenia is 40%-100% higher than among patients with other psychiatric diagnoses, and as much as three times higher than the prevalence of smoking in the general population (Hughes *et al.*, 1986; Goff *et al.*, 1992; Ziedonis *et al.*, 1994; Diwan *et al.*, 1998).

The very high rate of smoking among schizophrenic patients probably represents a form of self-medication with nicotine, which may improve certain symptoms of the disease as well as temporarily correct underlying neurobiological dysfunctions in the brain (Svensson *et al.*, 1990; Nomikos *et al.*, 2000; Simosky *et al.*, 2002; Martin *et al.*, 2004). Cigarette smoking may also reverse some of the mental side effects induced by typical antipsychotic drugs (Levin *et al.*, 1996). Thus, nicotine may also enhance cognition by its effect on attention e.g. by facilitating the release of neurotransmitters, such as acetylcholine, glutamate and dopamine.

1.7 The serotonergic system

Serotonin is found in many cells throughout the body and only 1-2% of the total amount is found in the brain. Functions associated with serotonin (5-HT) include feeding behavior, control of mood and emotions, sleep/wakefulness, temperature as well as various behavioral functions, such as hallucinations (Rang *et al.*, 2007).

1.7.1 Biochemistry

Neurons need to synthesize their own serotonin, since like other neurotransmitters it can not pass the blood-brain barrier. The precursor of serotonin is tryptophan, which primarily is derived from dietary proteins (see Figure 10). Tryptophan is actively taken up into neurons and the first step in the synthetic pathway of serotonin is hydroxylation of tryptophan to 5-hydroxytryptophan by tryptophan hydroxylase. 5-hydroxytryptophan is then decarboxylated to serotonin by a non-specific aromatic amino acid decarboxylase. Synthesized serotonin is stored in secretory granules. The rate limiting step in the serotonin synthesis is the availability of tryptophan and the activity of tryptophan hydroxylase. Serotonin is released into the synaptic cleft by Ca^{2+}-dependent exocytosis. The transmitter can serve as substrate for MAO, resulting in the formation of the metabolite 5-hydroxyindoleacetic acid (5-HIAA) and it can also be reduced to 5-hydroxytryptophol.

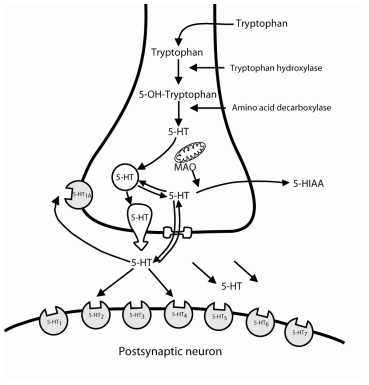

Figure 10. Schematic drawing of a serotonergic neuron. 5-HT: serotonin. Other abbreviations see text. Modified from (Cooper *et al.*, 2003).

1.7.2 Serotonergic pathways

Serotonergic pathways in the brain originate from nine nuclei, (named B1-B9) located along the midline of the brain stem. They project, via the medial forebrain bundle, to virtually all forebrain regions including, thalamus, basal ganglia, limbic system, hypothalamus, caudate-putamen, hippocampus and cortex (see Figure 11). The caudally positioned cells project to the cerebellum, medulla and the spinal cord (Azmitia & Segal, 1978; Cooper *et al.*, 2003; Rang *et al.*, 2007).

1.7.3 Serotonin receptors

Seven classes of serotonin receptors (5-HT_{1-7}) have been found to be expressed in brain. All 5-HT receptors are G-protein coupled receptors, except the 5-HT_3 receptor, which is a ligand-gated cation channel. The receptors 5-$HT_{1A, 1B, 1D, 2C, 3}$ and 5-HT_{5B} are both presynaptic and postsynaptic. While the 5-$HT_{1E, 1F, 2A, 2B, 4, 5A, 6}$ and 5-HT_7 receptors are exclusively expressed postsynaptically, the 5-HT_1 receptors are divided into subtypes A-F and are both pre-and postsynaptically located. These receptors activate a hyperpolarized inward current via inhibition of adenylyl cyclase. The 5-HT_{1A} receptors are found in high densities in the raphe nuclei, hippocampus and prefrontal cortex. The 5-HT_{1B} and 5-HT_{1D} receptors function as autoreceptors and mediate a negative feedback control of serotonin release. The 5-HT_{1B} receptors are expressed in the substantia nigra and globus pallidus, whereas the 5-HT_{1D} receptors are found in hippocampus, striatum and amygdala. The 5-HT_{1E} receptors are expressed in amygdala, caudate-putamen and cortex. The 5-HT_2 receptors are divided into subtypes A-C and exert an excitatory effect via the phospholipase C/IP3 pathway by reducing potassium exflux. The 5-HT_{2A} receptors are expressed in cortex and hippocampus and 5-HT_{2B} receptors are found in the cortex, whereas the 5-HT_{2D} receptors are found in the in medulla and hippocampus. The effects of the 5-HT_3 and 5-HT_4 receptors are excitatory and, the 5-HT_4 receptors mediate their effects through stimulation of adenylyl cyclase and increasing cAMP. The 5-HT_3 receptors are expressed in peripheral neurons and entorhinal cortex, whereas the 5HT_4 receptors are found e.g. in hippocampus. So far, relatively little is known about the functions and pharmacology of 5-HT_{5-7} receptors. The 5-HT_6 receptors are expressed in striatum, accumbens and cortex whereas the 5-HT_7 receptors are found in high density in thalamus, hypothalamus and amygdala (Cooper *et al.*, 2003).

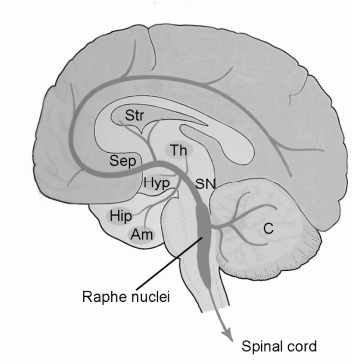

Figure 11. Serotonergic pathways in the human brain. Am: amygdalaoid nucleus; C: cerebellum; Hip: hippocampus; Hyp: hypothalamus; Sep: setum; SN: substantia nigra; Str: striatum. Modified from (Rang *et al.*, 1999).

1.7.4 Serotonin and schizophrenia

The involvement of serotonin in the pathophysiology of schizophrenia is supported by the psychomimetic effects of lysergic acid diethylamide (LSD; Gaddum & Hameed, 1954; Rinkel *et al.*, 1955), which now is known to act *inter alia* as a 5-HT_{2A} receptor agonist. Other findings, which proposed an altered serotonergic neurotransmission in schizophrenia, have been obtained in postmortem studies and include e.g. a decreased serotonin transporter density, an increased density of 5-HT_{1A} receptors (Hashimoto *et al.*, 1991; Burnet *et al.*, 1996; Gurevich & Joyce, 1997) and a decreased 5-HT_{2A} receptor density, needless to say, some of these effects might also be related to neuroleptic exposure (Mita *et al.*, 1986; Arora & Meltzer, 1991).

Indeed, several antipsychotic drugs have significantly higher affinity to 5-HT_2 receptors than to D_2 receptors.

1.8 The prefrontal cortex of the rat

The prefrontal cortex of the rat has been divided into medial, orbital and lateral parts. The medial prefrontal cortex consists of four main parts; the medial agranular (AGm), the anterior cingulate (AC), the prelimbic (PL), and the infralimbic (IL) cortex (Berendse & Groenewegen, 1991; Hoover & Vertes, 2007). Each part of the medial prefrontal cortex communicates with its adjacent regions and, with the possible exception of IL, each division interconnects with the others as well as brain regions projecting to the four parts of the medial prefrontal cortex. These regions include the amygdala, the midline thalamus, the VTA, the raphe nucleus, the LC (Hoover & Vertes, 2007) and the NAc (Kuroda *et al.*, 1996). These afferents thus include the dopaminergic, noradrenergic, cholinergic and serotonergic input in the medial prefrontal cortex. The very existence of a brain region in the rat that could be called prefrontal cortex has previously been questioned (Preuss, 1995). However, from an anatomical perspective the medial regions of the prefrontal cortex in rodents (e.g. prelimbic, infralimbic, and anterior cingulate) share similar patterns of efferent and afferent connectivity (e.g. afferents from the medial dorsal thalamic nucleus and dopaminergic innervations) with the dorsolateral prefrontal cortex in primates and humans (Ongur & Price, 2000). Furthermore, the rat medial prefrontal cortex also shares functional homology with the dorsolateral prefrontal cortex. Previous work has shown that lesions of the medial prefrontal cortex can impair both working memory and behavioral flexibility, behavioral deficits that are similar to the impairments observed after damage to the dorsolateral cortex in both primates and humans (Uylings *et al.*, 2003).

1.9 Treatment of schizophrenia

1.9.1 Pharmacotherapy

During the first half of the twentieth century opiates and sedatives as well as insulin coma were used to treat schizophrenia without producing consistent improvements in the disease (Ban, 2001). Surgery, mainly frontal lobotomy, was introduced in the 1930s and used until the late 1940s (Black, 1982). The clinical outcome of this treatment was poorly defined and the side effects were severe. Frontal lobotomy generated inertia, unresponsiveness, decreased attention span, blunted or inappropriate affect and disinhibition in the patients. Thus, this treatment could even cause a worsening of the disease (Mashour *et al.*, 2005). The discovery in the early 1950s, by Laborit, Delay and Deniker in France that the antihistaminic compound chlorpromazine had an antipsychotic effect, marked the beginning of the modern era and heralded a pharmacological revolution in the treatment of schizophrenia (Delay *et al.*, 1952; Bennett, 1998; Lopez-Munoz *et al.*, 2005). Before the introduction of modern pharmacotherapy, the great majority of schizophrenic patients suffered from a lifelong

psychosis, and the patients were hospitalized at enormous costs to the society. Following the introduction of chlorpromazine, numerous novel drug candidates were produced and tested in schizophrenic patients. These included drugs such as haloperidol, thiothixene but also reserpine. Reserpine which inhibits the storage of dopamine in the synaptic vesicles was initially considered as a viable alternative to chlorpromazine, but its popularity declined because of relatively low efficacy and many side effects. Today antipsychotic drugs are used to treat nearly all forms of psychiatric disorders, including schizophrenia, schizoaffecive disorder, affective disorder with psychosis as well as psychoses associated with organic mental disorders and drug-induced psychosis (Schatzberg & Nemeroff, 1998). Depending on their clinical profiles, antipsychotic drugs are usually classified as either typical or atypical antipsychotic drugs.

Examples of Typical antipsychotic drugs	
Chlorpromazine	Haloperidol
Examples of Atypical antipsychotic drugs	
Clozapine	Risperidone
Olanzapine	Sertindole
Quetiapine	Ziprazidone

1.9.2 Typical (first generation) antipsychotic drugs

Generally, positive symptoms are reduced in the majority of patients treated with typical antipsychotic drugs, whereas these drugs have less or even no effect on negative and cognitive symptoms, which may even deteriorate (Delay *et al.*, 1952; King, 1998). The clinical effect of the first typical antipsychotic drugs (e.g. chlorpromazine and haloperidol) was generally considered to be due to their ability to block dopamine receptors (see section 1.4.1). The development of PET and the use of receptor-selective ligands subsequently enabled clinicians to study the receptor occupancy of antipsychotic drugs in the brain of schizophrenic patients (see e.g. Farde *et al.*, 1988; Farde *et al.*, 1989; Farde *et al.*, 1992). Typical antipsychotic drugs show antipsychotic effect at a D_2 occupancy of 65-89% and e.g haloperidol generates a striatal D_2-occupancy of approximately 70% at clinically effective dosage (see section hypotheses 1.4).

Treatment with typical antipsychotic drugs is generally associated with a relatively high incidence of EPS. EPS are thought to be related to a high degree of D_2-receptor blockade in the nigrostriatal pathways, and at a D_2 occupancy > 80% the EPS liability increases substantially (Farde *et al.*, 1992). The EPS include: *i*) *acute dystonia* (which is caused by involuntary tonic contraction of skeletal muscles), *ii*) *akathisia,* (a subjective and objective motor restlessness often associated with even severe anxiety), *iii*) *parkinsonism* (akinesia, tremor and rigidity) (Marsden & Jenner, 1980; Hansen *et al.*, 1997), and *iv*) *tardive dyskinesia* (irregular stereotypical movements of the mouth, face and tongue, and choreoathetoid movements of fingers, arms, legs and trunk). Tardive dyskinesias usually occur after many years of use of antipsychotic medication (Sandyk *et al.*, 1993). Many antipsychotic drugs may also affect the tuberoinfundibular dopamine system, causing an increased prolactin secretion, resulting in side effects such as gynaecomastia, galactorré and sexual dysfunction.

1.9.3 Atypical (second generation) antipsychotic drugs

All atypical antipsychotic drugs are, like the typicals, antagonists at D_2 receptors, but the atypicals as a group have been considered to cause less EPS than the first generation antipsychotic drugs and, moreover, they have been claimed to improve negative and cognitive symptoms of schizophrenia better than the typicals. Apart from their affinity for D_2 receptors, most atypical antipsychotic drugs bind to a broad range of receptors including serotonergic, noradrenergic and other dopaminergic receptors. The first atypical antipsychotic drug was clozapine, which was introduced into clinical practice in the late 1960s. At this time, all existing antipsychotic agents were known to cause EPS (see above), but clozapine barely elicited catalepsy in rats, which originally caused scepticism as regards its utility as an antipsychotic drug. However, Hippius and others supported the development of clozapine in Germany (see Hippius, 1989). As a result, clozapine was marketed in a number of countries in Europe, but it was withdrawn from the market because of its ability to produce agranulocytosis (Idanpaan-Heikkila *et al.*, 1977), a side effect which was lethal in some cases. The drug was reintroduced in 1990 when it had been shown to be more efficacious than the typicals, especially in patients with treatment-resistant schizophrenia (Kane *et al.*, 1988a). Most atypical antipsychotic drugs e.g possess higher affinity for 5-HT_2 receptors than for D_2 receptors and some atypicals, such as clozapine, generate their clinical effect even at a D_2 occupancy of 45% (Farde & Nordstrom, 1992). Clozapine also displays a high affinity for D_3, D_4, α_1- and α_2, muscarinic and histaminic receptors. As mentioned above, most atypical antipsychotic drugs may not induce EPS to the same extent as the typical antipsychotics. However, some of the atypical antipsychotic drugs generate other severe side effects such as major weight gain and insulin resistance, which may lead to diabetes and cardiovascular disease, heavy sedation, hyperprolactinemia and even hypersalivation. However, in spite of such side effects, the use of clozapine may be justified if only because of its anti-suicidal effect in patients with schizophrenia (Kane *et al.*, 1988b; Meltzer, 2002).

1.9.4 Dopamine partial agonists (third generation antipsychotic drugs)

As mentioned above, all typical and atypical antipsychotic drugs are antagonists at D_2 receptors, and this effect is suggested to represent the major explanation to their effect on positive symptoms in schizophrenia. The development of partial agonists at dopamine receptors is based on the hypothesis that the symptoms of schizophrenia are to some extent caused by a combination of over-activity and under-activity in the dopamine pathways (see section 1.4.3). Thus, a dopamine stabilizer is a compound that may serve to normalize the overall dopaminergic transmission in the schizophrenic brain. The first clinically approved dopamine stabilizer was aripiprazole, which is a partial agonist at both D_2 and 5-HT_{1A} receptors, and which also possesses a modest antagonist affinity for 5-HT_2 and $5HT_7$ receptors, α_1-adrenoceptors and H_1 histaminergic receptors (Keck & McElroy, 2003). In addition to being a partial agonist at the D_2 receptors, aripiprazole has high affinity for the D_3 and D_4 receptors but negligibly affinity for D_1 and D_5 receptors (Keck & McElroy, 2003). The clinical effect of aripiprazole has now been demonstrated in a series of controlled clinical trials, and these studies

have shown sustained improvements of both positive and negative symptoms in schizophrenic patients. The antipsychotic effect of aripiprazole is generally comparable to those of typical and atypical antipsychotic drugs in patients with an acute relapse (Kane *et al.*, 2002; Potkin *et al.*, 2003). In contrast to many antipsychotic drugs, aripiprazole is not associated with increased prolactin secretion. Adverse events from different clinical studies include insomnia, agitation and anxiety. However, patients receiving aripiprazole experienced a lower incidence of akathisia, somnolence and EPS compared to those receiving haloperidol (Kane *et al.*, 2002), and reduced rates of akathisia, somnolence and tachycardia compared with those patients treated with risperidone (Chan *et al.*, 2007). Compared to olanzapine, aripiprizole causes less weight gain (Kujawa *et al.*, 2004).

1.9.5 Antipsychotic drugs and prefrontal glutamatergic neurotransmission

The development of improved pharmacological treatment of schizophrenia is currently expanding along several lines. One of these is to use various adjunct drugs (e.g. glycine, D-serine, glycine transporter inhibitors, anticonvulsant drugs, nACh receptor agonists, ampakines etc.) in combination with typical and atypical antipsychotic drugs. The purpose of this pharmacological approach is to target specific symptoms or symptom clusters, such as cognitive dysfunctions, which appear to represent the major determinant of treatment outcome in schizophrenia, and to diminish the risk of side effects since adjunct drugs may allow the use of lower doses of antipsychotic drugs and the side effects which are dose-dependent.

Therefore, in this thesis a set of preclinical studies using electrophysiological techniques *in vitro* was undertaken to investigate the effects of different add-on treatment strategies on NMDA-receptor mediated transmission in the rat medial prefrontal cortex.

2 SPECIFIC AIMS OF THE STUDY

- To examine the effect of nicotine on glutamatergic transmission in the medial prefrontal cortex, both when given alone or in combination with the $D_{2/3}$ antagonist raclopride or the D_4 antagonist L-745, 870.

- To investigate whether the glycine transporter (GlyT-1) inhibitor NFPS may modulate glutamatergic transmission in of the medial prefrontal cortex, both when given alone and in combination with risperidone or clozapine.

- To investigate the effect of topiramate on glutamatergic transmission in the medial prefrontal cortex, both when given alone and in combination with the $D_{2/3}$ antagonist raclopride or clozapine.

- To study the effect of the novel antipsychotic drug asenapine on: *i*) conditioned avoidance response, *ii*) catalepsy scores, *iii*) dopamine output in the medial prefrontal cortex and the nucleus accumbens, respectively, *iv*) dopamine output in the core and shell of the nucleus accumbens, and *v*) glutamatergic transmission in the medial prefrontal cortex.

3 MATERIALS AND METHODS

3.1 Animals

Male, albino Sprague-Dawley rats were used for voltammetry as well as electrophysiological recordings *in vitro* (average weight upon arrival: 270 [voltammetry]; 70g [electrophysiology]), whereas male Wistar rats were used for catalepsy, conditioned avoidance response (CAR) and microdialysis (average weight upon arrival: 200-250 g; Bantin & Kingman Universal, Sollentuna, Sweden). The animals were housed under standard laboratory conditions (i.e. under a controlled temperature [21ºC] and a relative humidity of 50-60%), with *ad libitum* access to food and water. For the microdialysis, voltammetry and electrophysiological experiments, the animals were kept on 12/12 h light/dark cycle (lights on at 6:00 AM), whereas for the behavioral tests, animals were maintained on a reversed 12/12 h light/dark cycle (lights off at 6:00 AM). Animals were acclimatised to their environment for at least five days before they were used in any experiments. All experiments were performed between 8:00 AM and 6:00 PM. All efforts were made to minimize the number of animals used and their suffering. All experiments were approved by, and conducted in accordance with, the Stockholm North Committee on Ethics of Animal Experimentation. Permit numbers: N7/00, N339/02, N93/05, N317/05, N335/05, and N338/05.

3.2 Drugs

Antipsychotic drugs

Clozapine	Novartis AG, Switzerland
Risperidone	Janssen Pharmaceutica Inc., Belgium
Asenapine	Organon Laboratories Ltd, Newhouse, United Kingdom

Catecholaminergic drugs

L-745,870	D_4 receptor antagonist, (3-[4-(4-chlorophenyl)piperazin-1-yl]-methyl-1H-pyrrolo[2,3b]pyridine), Merck, Germany
Raclopride	$D_{2/3}$ receptor antagonist, AstraZeneca, Sweden
SCH 23390	D_1 receptor antagonist, Tocris, UK
Pargyline	Non-selective monoamine oxidase inhibitor, Sigma-Aldrich Co, USA

Glutamatergic drugs

APV	NMDA receptor antagonist, 2-amino-5-phosphono-valeric acid, Sigma-Aldrich Co, USA

Glycine	Co-agonist at the NMDA receptor, Sigma-Aldrich Co, USA
NMDA	(*N*-methyl-D-aspartate), Sigma-Aldrich Co, USA

Anticonvulsant drugs

Topiramate	(2, 3:4, 5-bis-0-[1-methylethylidene]-β-D-fructo-pyranose Sulphamate), Johnson & Johnson, Pharmaceutical Research and Development, L.L.C., USA

Other drugs

(-)-Bicuculline methiodide	$GABA_A$ receptor antagonist, Sigma-Aldrich Co, USA
NFPS	Glycine transporter (GlyT-1) inhibitor; *N* [3-(4´-fluorophenyl)-3-(4´phenylphenylphenoxy) propyl] sarcosine, Sigma-Aldrich Co, USA
Nicotine di(+)tartrate	Sigma-Aldrich Co, USA
Tetrodotoxin	(TTX) Voltage-gated Na^+-blocker, Ascent Scientific, UK

Anaesthetics

Chloral Hydrate	Merck, Germany
Dormicum®	Midazolam, Roche, Stockholm, Sweden
Fluothane®	Halothane, AstraZeneca AB, Södertälje, Sweden
Hypnorm®	Fentanyl, citrate/ Fluanisone. Janssen-Cilag, Saunderton, UK

3.3 Electrophysiological experiments *in vitro* (Paper I-IV)

3.3.1 Background

Based on George Marmount's work 1947, Kenneth Cole developed the voltage-clamp technique to stabilize the membrane potentials of neurons for experimental purposes. It was used by Hodgkin and Huxley in the early 1950s in a series of experiments that revealed the mechanisms underlying the action potential. The voltage-clamp technique allows holding (clamp) a cell's membrane at a chosen value. This mode allows studying only ligand-gated ion-channels since clamping of the cell potential maintain voltage-dependent ion-channels inactivated. Thus, when the neuron is exposed to a drug which opens ion-channels, the effect of the ionic influx is counterbalanced by a current injected into the neuron by the amplifier. A fixed value, which makes it possible to measure ion-channel mediated currents across this membrane. By altering the extra – and intracellular compositions of buffers, ion-permeability and conductance properties of an ion channel may be determined (Schatzberg & Nemeroff, 1998).

3.3.2 Methodological considerations

Electrophysiological techniques *in vitro* have contributed to significant advances in understanding functional aspects of ion channels and how drugs modulate them.

There are advantages with electrophysiological techniques *in vitro*:

- Both intracellular and extracellular recordings are more stable because blood and breathing pulsations are absent.
- Visual control over electrode placement is achieved.
- The ionic composition of the microenvironment around cells may be controlled precisely.
- The concentration of drug in a slice or around a cell can easily be controlled.

3.3.3 Preparation of brain slices

The rats were decapitated under halothane anaesthesia and the brains were then rapidly removed and cooled in ice-cold Ringer's solution consisting of (in mM): NaCl 126, KCl 2.5, $CaCl_2$ 2.4, $MgCl_2$ 1.3, NaH_2PO_4 1.2, D-glucose 10, $NaHCO_3$ 18 (pH 7.4) and aerated by 95% O_2 / 5% CO_2. The brains were then cut coronally in order to produce 450 µm slices by using a vibratome (Vibroslice, Campden model MA 752, World Precision Instruments, Sarasota, FL, USA). The brain slices were removed from the vibratome and kept submerged in aerated Ringer's solution at room temperature for at least 1 hour to allow for recovery. A single slice was then transferred to the recording chamber (32˚C) in which it was held submerged between two nylon nets during the electrophysiological recordings. The chamber was continuously perfused with aerated Ringers solution at a flow rate of 2 mL/min.

3.3.4 Intracellular recordings

The electrophysiological recordings were performed in pyramidal neurons in layer V and VI (see figure 12) of the medial prefrontal cortex, located medial to the forceps minor. Pyramidal cells in these layers project to and receive input from subcotical areas (Kuroda *et al.*, 1996; Hoover & Vertes, 2007). Standard intracellular and single electrode voltage-and current-clamp recording techniques were used (Arvanov *et al.*, 1997; Arvanov & Wang, 1998). Recording electrodes were pulled from borosilicate glass capillaries (id: 0.58 mm, Clark Electromedical Instruments, Pangbourne, UK) by using a horizontal electrode puller (Model P-87, Sutter Instruments, San Rafael, CA, USA). Recording electrodes were filled with 2 M potassium acetate and used for intracellular recordings with an Axoclamp 2A amplifier (Axon Instruments, Foster City, CA, USA). An intracellular recording consisted of two parts, i.e. monitoring of the cell's membrane potential in the bridge mode and voltage-clamp recording of NMDA-induced currents in the pyramidal cell. Penetration of cells by a sharp electrode was performed blindly. Single-electrode voltage-clamp recordings were performed in the discontinuous mode with a sampling rate of 5-6.2 kHz, and acquired using digital/analogue sampling and a acquisition software (Clampex 9, Axon Instruments, Foster City, CA, USA).

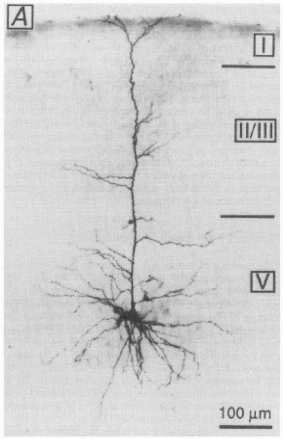

Figure 12. Photomicrograph of a biocytin-injected layer V pyramidal cell of rat medial cortex. Adaped from (Kawaguchi, 1993)

3.3.5 Characterization of pyramidal cells of the medial prefrontal cortex

In the rat prefrontal cortex about 60-70% of the neurons consist of pyramidal cells (Durstewitz *et al.*, 2000), and their sizes range from 12-36 µm and their soma can be found in layers V and VI (see Figure 12) (Kawaguchi, 1993). The electrophysiological criteria for distinguish presumed pyramidal cells from non-pyramidal neurons have been described previously (Arvanov *et al.*, 1997). In fact, the presumed pyramidal cells of the medial prefrontal cortex have relatively long spike duration (1-3 ms at half-maximum spike amplitude) and show pronounced spike frequency-adaptation in respons to constant-current depolarization pulses. As comparable, presumed non-pyramidal cells, characterized as interneurons, show brief spike duration (< 1 ms at half-maximum spike amplitude) and a general lack of spike-frequency adaptation.

3.4 NMDA-induced stimulation (Paper I-IV)

NMDA-induced currents were measured in the voltage-clamp mode (holding potential: -60 mV). During these experiments tetrodotoxin (TTX; 0.5 µM, to block the action potentials), glycine (1 µM, to enhance the NMDA-induced responses) and bicuculline (5 µM, to block the $GABA_A$ receptor responses) were routinely included in the perfusion buffer (i.e. Ringer's solution). The electrodes were filled with 2 M potassium acetate (the mean tip resistance was 64 MΩ, *n*= 176 cells). The drugs (clozapine, topiramate, SCH23390 and 2-amino-5 phosphonopentanoic acid [APV] were dissolved in dimethyl sulfoxide, whereas raclopride and asenapine were dissolved in purified water) were diluted in Ringer's solution (including TTX, glycine, and bicuculline). They were applied to the recording chamber by perfusion via a three-way tap system. NMDA (7.5 - 15 µM) was also diluted in Ringer's solution and applied via bath perfusion to induce inward currents (see Figure 13).

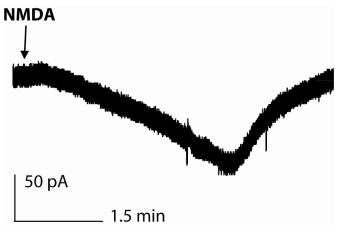

Figure 13. Representative trace illustrating the inward current, induced by NMDA, in a pyramidal cell of the medial prefrontal cortex.

3.5 Electrical stimulation of the forceps minor (Paper I and III)

Excitatory postsynaptic potentials (EPSPs) is a depolarization of the postsynaptic membrane potential caused by influx of positively charged ions into the postsynaptic cell. The stimulation of forceps minor involves increased release of excitatory amino acids (EEAs). The increased EAAs in turn activate postsynaptic non-NMDA- and (e.g. AMPA receptors) NMDA receptors. Thus, the EPSPs consist of an early, short duration component which has been shown to be blocked by the AMPA/kainate antagonist 6-cyano-7-nitroquino-xaline-2,3-dione (CNQX), and a prolonged late component which could be blocked by the NMDA receptor antagonist APV (Seamans *et al.*, 2001; Chen & Yang, 2002).

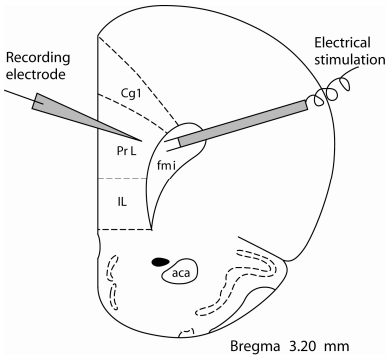

Figure 14. Schematic drawing of a representative section used for electrophysiological recordings of pyramidal cells in the medial prefrontal cortex. Shown are the positions of the stimulation and recording electrodes. Cg1: cingulated cortex, area 1; PrL: prelimbic cortex; IL: infralibic cortex; fmi: forceps minor; aca: anterior commisure, anterior part. Modified from (Paxinos & Watson, 1998)

The procedures for eliciting EPSPs by electrical stimulation of the forceps minor (white matter, see Figure 14) have been described elsewhere (Arvanov *et al.*, 1997; Chen & Yang, 2002). In order to record EPSPs, electrodes for intracellular recordings were filled with 2 M potassium acetate. The $GABA_A$ receptor antagonist bicuculline (2 μM) was included in the Ringer's solution. EPSPs, recorded in the current clamp mode, were elicited by passing rectangular current pulses (0.3 ms duration, 6-18 V) between the tips of a bipolar stainless-steel electrode placed in the medial part of the forceps minor close to the recording electrode (Arvanov & Wang, 1998). A train of six electrical pulses was delivered at a rate of 0.05 Hz before and after 5, 15, 25 and 35 min of drug application. The drugs tested were administered via bath application of Ringer's solution.

3.6 CATALEPSY (PAPER IV)

3.6.1 Catalepsy procedure

Catalepsy was observed in a dimly lit room by placing the animals on an inclined grid (60˚) for maximum 2.25 minutes. When a normal rat is placed in an unusual position, it will change its position within seconds, while a cataleptic rat will maintain its position for a longer of time (Sanberg *et al.*, 1988). The animals were allowed 30 seconds for adaptation on the grid before any observations started. Observations of the rats were performed at 30, 60 and 120 minutes after administration of drug or vehicle injection.

Score	Time
0	0-0.08
1	0.09-0.35
2	0.36-0.80
3	0.81-1.42
4	1.43-2.24
5	≥ 2.25

Table I. Catalepsy scores

The catalepsy was scored from 0 to 5, according to the time, in minutes (square root transformation) the rat remained immobile on the inclined grid (Ahlenius & Hillegaart, 1986), (see Table I). If the rat remained immobile for 0.08 minutes it was scored as 0, and so on. Each animal received only one treatment with drug.

3.6.2 Methodological consideration

Catalepsy is defined as the inability to correct an externally imposed and uncomfortable body posture, and this model is a tool to study neuropharmacological effects on extrapyramidal motor functions in laboratory animals (Arnt & Christensen, 1981; Wadenberg, 1996). Catalepsy in these animals is thought to be equivalent to antipsychotic drug-induced parkinsonism in humans (i.e. EPS). As mentioned earlier, EPS are associated with typical antipsychotic drugs and their high degree of D_2 receptor blockade in the dopaminergic nigrostriatal pathway (see section 1.2.2 and 1.9.2). Although catalepsy is a robust behavioural method many variables may influence the cataleptic effect (Sanberg *et al.*, 1988). For example, auditory and visual stimuli as well as handling the animals gently are important variables in catalepsy testing.

3.7 Conditioned avoidance response (CAR; Paper IV)

3.7.1 CAR procedure

The animals were transferred from the animal facility to the laboratory 1 hour before the CAR tests started, and were housed in a ventilated cabinet between observations. Rats were trained 15 minutes per animal and day for 5 consecutive days in a conventional shuttle-box (530 mm × 250 mm × 225 mm) divided into two equal compartments by a partition with an opening. Only animals reliably performing at a level of > 90% avoidance were included in the studies and tested individually [the same animals were tested repeatedly serving as their own controls in a change over design (Li, 1964)] The apparatus was equipped with photo cells, automatically registering the position of the rat in relation to the opening, and a grid floor connected to a high resistance power supply. Rats were trained to avoid the unconditioned stimuli (UCS), an intermittent electric shock in the grid floor of approximately 0.3 mA (inter shock interval 2.5 seconds, shock duration 0.5 seconds), by moving into the opposite compartment within 10 s of an 80 dB white noise (white noise generator; Lafayette Instruments, Lafayette, IN, USA), which is used as conditioned stimuli (CS). White noise is a (hissing-like) type of noise that is

Behavioral variable	
Avoidance	Response to CS within 10 s
Escape	Response to CS and UCS
Escape failure	If the rat was unable to respond to the shock by moving into the opposite compartment within 50 seconds the trial was terminated
Inter-trial crosses	Movement between compartments between trials

Table II.

produced by combining equal intensity sounds of all different frequencies to form a broadband spectrum type of sound. The behavioral variables recorded were avoidance, escape, escape failure (Salmi *et al.*, 1994) and inter-trial crosses (see Table II). If the rat did not respond within 50 seconds, the trial was terminated (escape failure). Experimental manipulations were always preceded by a pre test and experiment sessions, lasting 10 minutes, were conducted 20, 90 and 240 minutes after injection of drug or vehicle.

3.7.2 Methodological considerations

The CAR test has been used since the 1950s and is a very sensitive test for detecting antipsychotic effect of drugs or drug combinations. Even though this model has been used for about 50 years, the mechanism underlying the CAR behavior remains unknown. However, local application of a selective dopamine D_2 antagonist suggests that a suppression of CAR behavior is mediated by inhibition of the mesolimbic dopamine pathway (Wadenberg *et al.*, 1990). Furthermore, it was recently shown that antipsychotic-like suppression of CAR by antipsychotic drugs occur at the same level of D_2 occupancy in the striatum (65-70%) that is usually needed for therapeutic response to antipsychotic treatment in schizophrenic patients (Kapur *et al.*, 2000; Wadenberg *et al.*, 2001). In this test, rats are presented to a CS i.e. a noise, to make an active response to avoide or escape an aversive UCS such as an electric foot shock (Wadenberg & Hicks, 1999). Administration of drugs with antipsychotic potential result in a deficit of the response. The differential effect of drugs with antipsychotic potential on avoidance behaviour distinguish these drugs from other avoidance disrupting drugs such as e.g. benzodiazepines or barbiturates that show overlapping dose-effect relations for avoidance and escape behaviour (Arnt, 1982; Wadenberg & Hicks, 1999). A selective suppression of avoidance but not escape behavior is thus interpreted as a strong indication of antipsychotic-like activity. The appearance of escape failures is indicative of unwanted and unspecific side effects such as sedation or motor dysfunction and excludes, in principle, a specific antipsychotic activity of a drug.

In vivo microdialysis (Paper IV)

3.7.3 Microdialysis surgery

The rats were anaesthetized with a cocktail of Hypnorm® (0.315 mg/mL fentanyl citrate and 10 mg/mL fluanisone) and Dormicum® (5 mg/mL midazolam) diluted in distilled water (1:1:2; 5 mL/kg, i.p.), mounted in a stereotaxic instrument and implanted with a probe in the medial prefrontal cortex, NAc or lateral striatum (anterior-posterior [AP]: +2.8 mm, +1.4 mm, +0.7 mm; medial-lateral [ML]: -0.6 mm, -1.4 mm, -3.5 mm; dorsal lateral [DL]: -5.2 mm, -8.2 mm, -6.2 mm) respectively, relative to bregma and dural surface, according to the Paxinos and Watson rat brain atlas (Paxinos & Watson, 1998).

3.7.4 Microdialysis procedure

Dialysis occurred through a semipermeable membrane (Filtral AN69, Hospal Industrie, Meyzieu, France) with an active surface length of 2.25 mm for the NAc, 4 mm for the medial prefrontal cortex and 3 mm for striatum. Dialysis experiment was conducted approximately 48

hours after surgery in awake, freely moving rats. The dialysis probes were perfused with a physiologic salt solution containing 147 mM NaCl, 3.0 mM KCl, 1.3 mM $CaCl_2$, 1.0 mM $MgCl_2$, 1.0 mM $NaHPO_4$ (Apoteksbolaget, Stockholm, Sweden) pH: 7.4, at a rate of 2,5 μL/min set by a microinfusion pump (Harvard Apparatus, Holliston Massachusetts; USA). The dialysate samples were collected over 30 or 15 min intervals for the medial prefrontal cortex or Nac and striatum, respectively. Online quantification of dopamine and metabolites in the dialysate was accomplished by a high performance liquid chromatography (HPLC) system coupled to electrochemical detection (Bioscience, Chelsford, MA, USA), with a detection limit of approximately 0.2 fmol/min. The loading and injecting modes of the injector (Valco Instruments, Houston, Texas, USA) were directed by a computerized system with Totalcrom WS 6.3 software (Perkin Elmer, Wellesley, MA, USA). The separation of dopamine, DOPAC, HVA and hydroxyindolacetic acid (5-HIAA) were achieved by a reversed phase liquid chromatography. The mobile phase for dopamine detection consisted of a 55 mM sodium acetate buffer, pH: 4.0 (adjusted with glacial acetic acid) with 12% methanol and 0.55 mM octanesulfonic acid. It was delivered by a HPLC pump (Model 2150, Pharmacia LKB, Sweden) on a C-18 column (Nucleosil 150/75 × 4.6 mm, 5 μm) at a flow rate of 0.8 mL/min. After separation, the analysate was passed through a guard cell with an applied oxidizing potential of 50 mV to reduce baseline noise from other electroactive compounds. Samples were then quantified by sequential oxidation and reduction in a high-sensitivity analytical cell (5011; ESA Biosciences, Chelmsford, MA, USA) that was controlled by a potentiostat (Coulochem II model 5200; ESA Bioscience, MA, USA) with applied potentials of 400 mV and -200 mV for detection of metabolites and dopamine, respectively, in NAc and striatum, and 250 mV and -300 mV for detection of metabolites and dopamine in the medial prefrontal cortex, respectively. Chromatograms were both printed on a two-pen chart recorder (Kipp & Zonen, Delft, The Netherlands) and recorded by the computer. Injection of the antipsychotic drug was performed after a stable outflow (<10% variation) of dopamine (DOPAC, 5-HIAA, HVA). The dopamine output is expressed as percent of baseline. Baseline (= 100%) was defined as the average of the last two (medial prefrontal cortex) or four (NAc and striatum) pre-injection values. Upon completion of dialysis experiment, animals were sacrificed with an overdose of anaesthetics, and their brains were collected and preserved in a 10% formalin / 25% sucrose solution for at least 2 days.

3.7.5 Methodological considerations

Microdialysis is a technique that is designed for sampling the chemistry of human or animal tissue over time and the purpose of the technique is to mimic the function of a small blood vessel (Ungerstedt, 1991). Through a dialysis membrane inserted into the tissue of interest, the technique allows substances to both be delivered to the tissue as well as to be sampled from the tissue. Here we have used the technique for chemically detect the composition of extracellular fluid in the medial prefrontal cortex, NAc or lateral striatum after drug injection. The advantages of this technique are that they can be performed in awake and freely moving rats and that substances can be monitored continuously for a prolonged period of time. One of the disadvantages with the technique is the relatively poor recovery of neurotransmitters over the membrane, resulting in a relatively poor time resolution (15-30 minutes are required depending on the neurotransmitter measured and the anatomical area).

Furthermore, the insertion of the probe causes localised tissue damage and the relatively large size of the probe (~300 µm in diameter) makes is hard to discriminate between small regions.

3.8 *In vivo* voltammetry (Paper IV)

3.8.1 Surgery

Rats were pre-treated with the non-selective MAO inhibiotor pargyline (75 mg/kg, i.p., 90-120 minutes before recording) and anaesthetized with chloral hydrate (400 mg/kg, i.p.). To achieve free respiration a tracheal catheter was inserted. In addition, a jugular and a femoral vein catheter for i.v. administration of drugs and continuous infusion of chloral hydrate, respectively, were inserted. The animals were kept under surgical anaesthesia throughout the experiment and normal body temperature was maintained by using thermostatically controlled electric heating pad. Rats were mounted in a stereotaxic instrument (Unimecanique, France) with the incisor bar 3 mm under the horizontal plane passing through the interaural line. The skull was exposed and a hole was drilled above the NAc. The dura mater was incised and the pia mater was punctured by means of a used electrode. Electrodes were inserted in either the NAc core (AP: +1.7 mm, ML: +1.6 mm relative to bregma) or the NAc shell region (AP: +1.6 mm, ML: +0.8 mm relative to bregma, see Figure 15) according with the atlas of Paxinos and Watson (Paxinos & Watson, 1998).

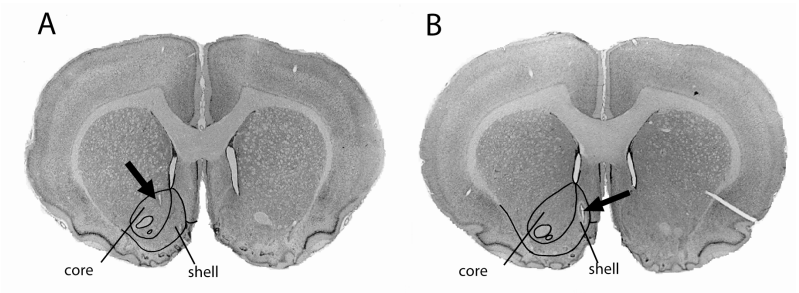

Figure 15. Photomicrographs showing the site of the electrode position in the (A) the core and (B) the shell subdivision of the NAc

3.8.2 Carbon filter electrodes and electrochemical measurement

Carbon filter electrodes (active portion 12 µm thick and 500 µm long) were prepared and treated as described by Gonon *et al* (Gonon *et al.*, 1984) except that the amplitude of the first treatment was + 2.5-2.7 V for 20 seconds, then 0.7 V (cathodic potential) for 5 seconds and +1.5 V for 5 seconds, successively. The activation of the electrode surface by an electrochemical treatment improves the selectivity and sensitivity of the measurement, i.e. allows the separation of the ascorbic acid from the catechols, whereas the pargyline pre-treatment of the animals prevents the formation of DOPAC, thus minimizing the interference of DOPAC with the catechol peak (Gonon, 1988). The tip of the prepared and treated electrode was placed 6.5-7.0 mm below the cortical surface and the catechol oxidation current was monitored by a pulse voltammetric system (Biopulse, SOLEA Tacusell, France). Differential

normal pulse voltammetry (Adams, 1990) was used to record voltammograms with the parameters previously described (Gonon, 1988). The biopulse system was connected to a computer with the chromatography software and data acquisition system AZUR version 4.0 (Datalys, France) which the peak area was calculated. The average of ten 1-minutes samples before the first injection was used as a baseline. After a stable baseline had been achieved and subsequently recorded for at least 10 minutes, the animals received an injection (i.v.) of vehicle to exclude non-drug-specific effects and 10 minutes later the drug was injected. For each experiment values were expressed as percent of baseline. The overall dopamine output was measured as the mean ± standard error of mean (S.E.M.) percent change from baseline over 15 minutes following drug injection. At the end of each experiment, an electrolytic lesion (5V, 5 seconds) was made through the carbon fibre electrode for histological verification of the recording site. Upon completion of the experiment the animals were sacrificed and their brains were stored in a 25% sucrose / 4% formaldehyde solution.

3.8.3 Methodological considerations

The advantages of this technique are that they can be performed in local areas of the brain (e.g. core and shell of the NAc) with higher spatial (electrode 12µm in diameter) and temporal resolutions compared to *in vivo* microdialysis.

3.9 Histological verification of probe and electrode placements experiments *in vivo*

Each brain taken from microdialysis and voltammetry experiments was cut in 50µM sections of the NAc, striatum or medial prefrontal cortex on a microtome (SLEE, Mainz, Germany), Nissl-stained, and examined under a microscope for electrode placement. Only animals with correct electrode positioning were included in the study.

3.10 Data analysis and statistics

3.10.1 Electrophysiology *in vitro*

The effects of the drugs or drug combinations on the NMDA-induced currents were calculated by dividing the NMDA-induced currents after the bath application of the drugs by the control NMDA-induced currents. Statistical comparison of data was performed by using paired t-test or Student´s t-test, and, for multiple comparisons, one-way analysis of variance (ANOVA) followed by the *post hoc* Tukey HSD test (study II, III and IV) or by LSD *post-hoc* comparison test (study I)

3.10.2 Behavioral studies (CAR and Catalepsy)

The statistical evaluation of behaviour data was performed by Friedman two-way ANOVA, followed by Wilcoxon matched-pairs signed-ranks test (CAR) or the Kruskal-Wallis one-way ANOVA, followed by Mann-Whitney *U* test (catalepsy).

3.10.3 Microdialysis *in vivo*

Statistical evaluation of microdialys data over time was performed by means of a two-way (treatment × time) ANOVA for repeated measures followed by a planned comparison test, whereas overall effects presented as area under the curve were statistically evaluated by means of a one-way (treatment) or a two-way (treatment × area) ANOVA for a multiple categorical variables followed by Neuman-Keuls test

3.10.4 Voltammetry *in vivo*

Voltammetry data were statistically evaluated by a t-test for dependent samples.

In all statistical measures, $p < 0.05$ was considered to be significant.

4 RESULTS AND DISCUSSION

4.1 Electrophysiological characterization of pyramidal cells in the medial prefrontal cortex

In the rat prefrontal cortex about 60-70% of the neurons are pyramidal cells (Durstewitz *et al.*, 2000) positioned in layers V and VI (Kawaguchi, 1993). Electrophysiological and morphological studies of neurons in cortical areas have revealed four types of pyramidal cells based on their characteristic firing patterns. They can be divided into regular spiking, intrinsic spiking, repetitive oscillatory bursting and intermediate cells (Yang *et al.*, 1996). In rodents and in primates, cortical pyramidal neurons form long recurrent collateral connections with neighbouring pyramidal cells, and excitation of these connections is thought to be one of the essential mechanisms underlying the generation of persistent neuronal activity in the prefrontal cortex (Levitt *et al.*, 1993; Douglas *et al.*, 1995; Chen & Yang, 2002), which in turn is required for prefrontal functions including the cognitive function working memory (Camperi & Wang, 1998; Wang, 1999; Shu *et al.*, 2003).

Parameters to distinguish pyramidal cells from non-pyramidal cells (e.g. fast spiking cells, glial cells, or interneurons) have been described in the Materials and Methods section (see section 3.3.5). Presumed pyramidal cells of the layers V and VI in the rat medial prefrontal cortex exhibited a mean membrane potential of -77 ±1 mV (n=193), an action potential amplitude of 75 ± 1 mV (n=190), an afterhyperpolarization potential of 5.1 ± 0.3 mV (n=183), a spike frequency adaptation of 2.3 ± 0.3 (n=174), and a spike half width of 4.6 ± 0.7 ms (n=119). These results are comparable to those previously published (Arvanov *et al.*, 1997; Ninan *et al.*, 2003b).

4.2 Atypical, but not typical antipsychotic drugs markedly enhance NMDA-induced currents in the medial prefrontal cortex

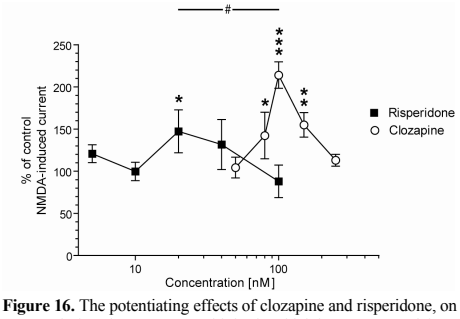

Figure 16. The potentiating effects of clozapine and risperidone, on NMDA-induced currents were biphasic which is a property of the effect of all tested atypical antipsychotic drugs on these currents in the medial prefrontal cortex (Ninan *et al.*, 2003b). **P* <0.05, ** *P* <0.01, ****P* <0.001 (paired *t*-test different from the control NMDA-induced current). #P < 0.05 (Student's *t*-test; significantly different from the maximal effect of clozapine on the NMDA-induced currents). The holding potential was -60 mV

As mentioned in the introduction 1.4.2, the glutamate hypothesis of schizophrenia proposes a relationship between hypoactive glutamate neurotransmission, particularly NMDA receptor hypofunction and the pathophysiology of schizophrenia. Wang and associates have previously reported that atypical (e.g. clozapine and risperidone), but not typical (e.g. haloperidol and raclopride) antipsychotic drugs markedly facilitate NMDA receptor-mediated

glutamatergic transmission in the medial prefrontal cortex, an effect which was hypothesized to contribute to the ability of the atypical antipsychotic drugs to improve negative symptoms and cognitive dysfunctions in schizophrenia (Arvanov *et al.*, 1997; Ninan *et al.*, 2003b).

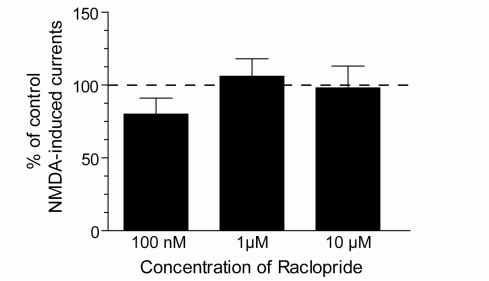

Figure 17. Raclopride had no effect on the NMDA-induced currents in the pyramidal cells of the medial prefrontal cortex. The holding potential was -60 mV.

Bath perfusion of NMDA induced inward currents (10 µM NMDA; 44±4 pA, *n*=58) in the pyramidal cells of the medial prefrontal cortex. As reported previously (Arvanov *et al.*, 1997; Ninan *et al.*, 2003a), clozapine and risperidone (Figure 16), but not raclopride (Figure 17) potentiated the NMDA-induced currents in medial prefrontal cortex. As also seen in Figure 16, the concentration-response curves of their effects on the NMDA-evoked currents were biphasic.

4.3 Paper I. Addition of nicotine to the $D_{2/3}$ antagonist raclopride or the weak D_4 antagonist L-745,870 augments NMDA-induced currents in pyramidal cells of the medial prefrontal cortex

As mentioned in section 1.6.4, schizophrenic patients are heavy smokers (Hughes *et al.*, 1986), and it has been proposed that the intense tobacco consumption may represent a form of self-medication (Svensson *et al.*, 1990; Freedman *et al.*, 2000; Nomikos *et al.*, 2000) by nicotine. Indeed, nicotine has been found to reduce both cognitive and negative symptoms in schizophrenic patients treated with typical antipsychotic drugs (Hughes *et al.*, 1986; Simosky *et al.*, 2002; Martin *et al.*, 2004). Interestingly, clozapine decreases smoking in these patients (McEvoy *et al.*, 1995a). Clinical evidence indicates that clozapine and other atypical antipsychotic drugs possess superior efficacy over typical antipsychotic drugs especially against cognitive and negative symptoms (see e.g. Davis *et al.*, 2003). These effects may in part be explained by their ability to markedly enhance prefrontal dopamine release (Moghaddam & Bunney, 1990; Nomikos *et al.*, 1994) and glutamatergic transmission (Arvanov *et al.*, 1997; Ninan *et al.*, 2003b). Previous data also show that nicotine may increase prefrontal dopamine output, an effect that appears enhanced with repeated administration (Nisell *et al.*, 1996). Moreover, both electrophysiological and biochemical data support a glutamate releasing effect of nicotine in the brain, including the VTA (Schilström *et al.*, 2000) and the prefrontal cortex (Gioanni *et al.*, 1999).

In this paper (I), we have, using electrophysiological techniques, explored the effects, of nicotine on NMDA-mediated transmission in the medial prefrontal cortex, both when given alone and in combination with either the $D_{2/3}$ antagonist raclopride or the weak D_4 antagonist L-745,870. The concentrations of nicotine in the electrophysiological experiments correspond to the serum concentrations in humans 5 minutes after smoking 1-3 cigarettes, and these

concentrations are thus within a clinically relevant range (Benowitz *et al.*, 1988; Benowitz *et al.*, 1989).

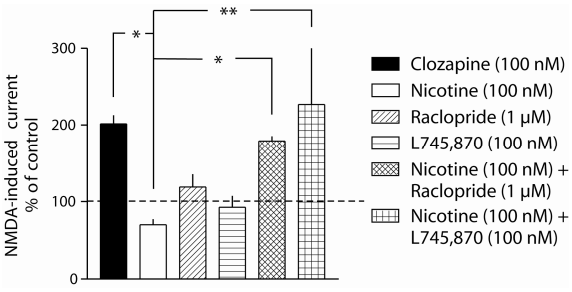

Figure 18. Histogram showing the effect of clozapine, nicotine, raclopride, or L-745,870 alone, as well as the combinations nicotine and raclopride and nicotine and L-745,870 on the NMDA-induced currents in the pyramidal cells in the medial prefrontal cortex. **P* <0.05, ** *P* <0.01, ****P* <0.001 (one-way ANOVA followed by LSD post-hoc comparison). The holding potential was -60 mV.

No effect was seen on NMDA-induced currents in pyramidal cells of the mPFC with raclopride, L-745, 870 or nicotine when given alone (see Figure 18). However, the combination of either raclopride or L-745,870 with nicotine generated a facilitating effect on the NMDA-induced currents, which was of the same magnitude as the effect of clozapine on these currents (see Figure 16 and 18).

The mechanism underlying the facilitating effects of these drug combinations on the NMDA-induced currents in the medial prefrontal cortex may tentatively involve prefrontal dopamine, since previous studies have demonstrated that nicotine and the D_4 antagonist PNU 1013876 similarly to clozapine and other atypical antipsychotic drugs, augment the dopamine output in the medial prefrontal cortex (Nisell *et al.*, 1996; Broderick & Piercey, 1998). D_2 antagonists, such as haloperidol and raclopride, may also increase prefrontal dopamine, but this effect is much smaller than that of clozapine (Moghaddam & Bunney, 1990; Nomikos *et al.*, 1994). Earlier data has shown that nicotine activates dopamine cell firing rate as well as burst firing (Grenhoff *et al.*, 1986) and that nACh receptors in the VTA are critically involved in the stimulatory effect of systemic nicotine on terminal dopamine release (Nisell *et al.*, 1994; Schilström *et al.*, 1998). However, recent evidence indicates that nicotine may also influence prefrontal dopamine release by means of a TTX-insensitive modulation of the dopamine transporter via nACh receptors (containing the α4 and β2 subunits) located on dopaminergic afferents in the prefrontal cortex (Drew *et al.*, 2000; Drew & Werling, 2003). Our data propose that the effects of nicotine, raclopride or L-745,870 alone on prefrontal dopamine may be insufficient to enhance NMDA-mediated transmission. However, the combination of either a D_2 antagonist or a D_4 antagonist with nicotine might generate a synergistic effect on dopamine output in the medial prefrontal cortex, which in turn may cause a clozapine-like facilitation of the NMDA-induced currents in the pyramidal cells.

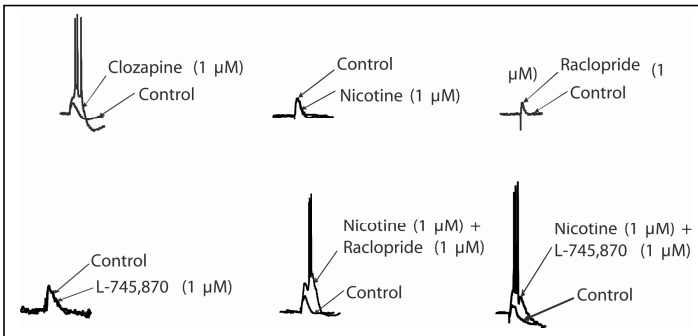

Figure 19. Representative traces illustrating the effect of of clozapine, nicotine, raclopride, or L-745,870 alone, as well as the combinations nicotine and raclopride and nicotine and L-745,870 on EPSPs in pyramidal cells in the medial prefrontal cortex.

To further understand the mechanisms of these drugs on glutamatergic transmission, we investigated the effects of nicotine, raclopride or clozapine alone, as well as the combinations of nicotine and raclopride or nicotine and L-745,870, on electrically evoked EPSPs

Consistent with earlier results, clozapine markedly produced bursts of action potentials, on polysynaptically mediated evoked EPSPs in pyramidal cells of the medial prefrontal cortex (Chen & Yang, 2002; Ninan *et al.*, 2003b). When administrated alone neither nicotine, raclopride nor L-745,870 produced any effect, while the combinations of nicotine and raclopride as well as nicotine and L-745,870 both mimicked the effect of clozapine on these EPSPs (see Figure 19). The potentiating effect on the EPSPs by these drug combinations are mediated by NMDA receptors, since they were blocked by the selective NMDA receptor antagonist APV. Thus, clozapine, as well as the combination of nicotine and raclopride or nicotine and L-745,870, may facilitate NMDA receptor mediated network interactions between interconnected pyramidal cells.

Moreover, recent studies suggest an interaction between NMDA receptors and D_4 receptors. Thus, electrophysiological studies have demonstrated that D_4 activation decreases NMDA-mediated currents in the prefrontal cortex. (Wang *et al.*, 2003). A D_4 antagonist might thus tentatively produce the opposite effect and enhance these currents. However, since we did not observe any enhancement of NMDA currents or the EPSPs by the weak D_4 antagonist L-745,870 when given alone in the present experiments, such enhancement, if it was to occur, may be dependent on higher concentrations of the drug.

Understanding the interaction between nicotine and antipsychotic drugs is a clinically important issue, since, as mentioned earlier, schizophrenic patients are frequently heavy smokers, although depending on their antipsychotic medication they their smoking habits may vary considerably; thus whereas clozapine appears to decrease smoking in these patients, haloperidol rather increase smoking (McEvoy *et al.*, 1995b). Our results generally suggest, that the synergistic effects of combining nACh receptor agonists with dopamine D2-like receptor antagonists be useful in the treatment of schizophrenia and may serve to improve negative and cognitive symptoms in these patients.

4.4 Paper II. Differential effects of the GlyT-1 inhibitor NFPS on NMDA-induced currents in the medial prefrontal cortex.

Therapeutic approaches in schizophrenia which aim selectively at modulating glutamatergic neurotransmission, especially the NMDA receptor-mediated transmission, are relatively new. Recent clinical data indicate improvement of particularly negative symptoms in schizophrenic patients when co-agonists at NMDA receptors, e.g. glycine (Heresco-Levy *et al.*, 1996; Leiderman *et al.*, 1996; Heresco-Levy *et al.*, 1999; Heresco-Levy & Javitt, 2004), D-serine (Tsai *et al.*, 1998) and D-alanine (Tsai *et al.*, 2006), are added to typical and some atypical antipsychotic drugs. Instead of adding co-agonists to enhance NMDA receptor function, synaptic levels of glycine can be elevated pharmacologically by blocking glycine transporters (GlyTs). The GlyTs belong to the family of sodium/chloride dependent transporters, which include GlyT-1 and GlyT-2 (Aragon & Lopez-Corcuera, 2003). Using hybridization and immunocytochemistry studies, it has been found that cells expressing GlyT-1 mRNA are expressed throughout the CNS (Eulenburg *et al.*, 2005), but enriched in the forebrain (Smith *et al.*, 1992; Borowsky *et al.*, 1993) and in areas with high expression of NMDA receptors, which is consonant with a role in the control of the local glycine concentration in the vicinity of the NMDA receptors (Aragon & Lopez-Corcuera, 2003). The GlyT-1 is expressed primarily in glial cells adjacent to the glutamatergic synapses, while the GlyT-2 is co-localized with inhibitory glycine receptors (Aragon & Lopez-Corcuera, 2003; Depoortere *et al.*, 2005) and expressed in the spinal cord, brain stem and cerebellum (Eulenburg *et al.*, 2005).

Clinical studies by Tsai *et al* (Tsai *et al.*, 2004) and Lane *et al* (Lane *et al.*, 2006) have shown that a GlyT-1 inhibitor, N-methylglycine (sarcosine) may improve positive, negative and cognitive symptoms in schizophrenic patients treated with risperidone but not with clozapine (Lane *et al.*, 2006).

Against this background, using intracellular recordings *in vitro*, we tested the effect of the GlyT-1 inhibitor *N* [3-(4'-fluorophenyl)-3-(4'-phenylphenylphenoxy) propyl] sarcosine (NFPS) on NMDA-induced currents in pyramidal cells of the rat medial prefrontal cortex, both when given alone and in combination with either risperidone or clozapine.

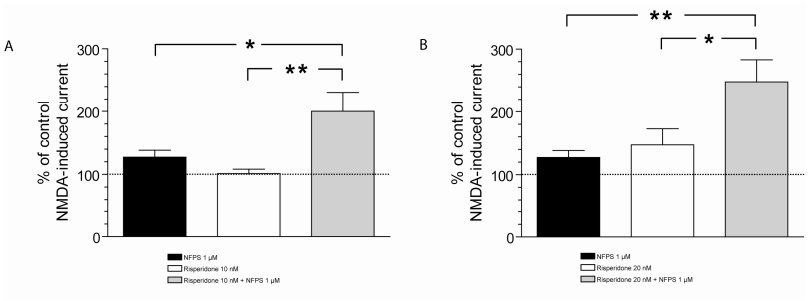

Figure 20. Inhibition of GlyT-1 by NFPS markedly enhanced the effect of both a submaximal and the maximal effective concentration of risperidone, up to the level of te maximal effect of clozapine. * P <0.05, ** P <0.01 (one-way ANOVA followed by Tukey HSD test). The holding potential was -60 mV.

Clinically effective doses of risperidone in schizophrenic patients have been estimated to yield plasma levels of about 2-78 nM (Spina *et al.*, 2001), and around 445-3000 nM (Spina *et al.*, 2000) in patients treated with clozapine. In this study we found that NFPS alone had a small but significant potentiating effect on the NMDA-induced currents in the presence of high levels of extracellular glycine. When NFPS was combined with risperidone, we observed an enhanced effect on the NMDA-induced currents of both a submaximal (10 nM) and a maximal (20 nM) concentration of risperidone (see Figure 20). Interestingly, the maximal effect of this combination was larger than the maximal effect induced by clozapine on these currents (see Figure 16). Moreover, blocking the GlyT-1 could not further enhance the submaximal (80 nM) or the maximal (100 nM) concentration of clozapine (Figure 21).

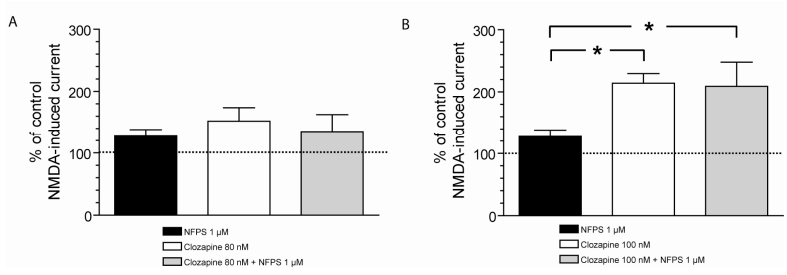

Figure 21. Inhibition of GlyT-1 by NFPS did not potentiate either the effect of a submaximal or the maximal effective concentration of clozapine. **P* <0.05 (one-way ANOVA followed by Tukey HSD test). The holding potential was -60 mV.

Our experimental finding in this study, that the effect of risperidone on NMDA receptor mediated currents could be enhanced with the GlyT-1 inhibitor NFPS may provide an explanation to, the clinical observation that the GlyT-1 antagonist sarcosine may improve negative and cognitive symptoms in patients treated with risperidone (Tsai *et al.*, 2004). Moreover, the lack of effect of NFPS on the clozapine-induced enhancement of NMDA receptor currents, provides an experimental correlate to the absence of ameliorating effect of sarcosine in patients receiving clozapine (Lane *et al.*, 2006).

The fact that we were not able to further enhance the effect of clozapine on the NMDA-induced currents indicates that clozapine and risperidone both quantitatively and qualitatively may differ in their effects on glutamatergic transmission in the prefrontal cortex. Our data and previous preclinical studies indicate that clozapine, but not risperidone, induces saturation of the GlyB site of the NMDA receptor. This saturation may be explained by several mechanisms. Clozapine may enhance synaptic levels of glycine by inhibiting system A glutamine transporter sites (SNAT1) for neuronal uptake of glycine (Schwieler *et al.*, 2004; Javitt *et al.*, 2005). Another possibility might be direct blockade of the GlyT-1 transporter, but this mechanism is unlikely to occur *in vivo,* since the IC-50 value of clozapine for this protein is 100 µM (Williams *et al.*, 2004). Our conclusion is supported by clinical data showing that the partial NMDA agonist D-cycloserine does not improve negative symptoms when added to clozapine and, in fact some negative symptoms even worsened (Goff *et al.*, 1999a). Interestingly, reduced plasma glycine levels have been observed both in unmedicated schizophrenic patients (Sumiyoshi *et al.*, 2004) and in patients treated with typical antipsychotics, as well as risperidone or olanzapine. However, a similar reduction of plasma glycine levels was not

observed in those patients treated with clozapine, suggesting that clozapine may also modulate peripheral glycine levels (Neeman *et al.*, 2005). Indeed, other mechanisms by which clozapine may modulate NMDA receptor-mediated neurotransmission via the glycine site have also been suggested (Javitt, 2006). GlyT-1 transporters are expressed in the forebrain and microdialysis data have shown that the GlyT-1 inhibitor SSR504734 increases the levels of both glycine and dopamine in the prefrontal cortex (Depoortere *et al.*, 2005). This finding may have bearing on our observation that the GlyT-1 inhibitor NFPS enhanced NMDA receptor currents by even given alone. Taken together, our experimental findings therefore suggest the notion that GlyT-1 inhibitors may provide a clinically useful adjunctive treatment strategy in schizophrenic patients receiving both typical and atypical antipsychotic drugs with the exception of clozapine.

4.5 Paper III. Differential effects of topiramate on prefrontal glutamatergic transmission when combined with raclopride or clozapine

Anticonvulsant drugs (such as valproate and carbamazepine) were originally introduced as mood stabilizers for the treatment of bipolar disorder but are nowadays used in a wider spectrum of mental disorders, for example as adjuvant treatment in schizophrenia. Topiramate is a relatively new anticonvulsant drug which has been explored in the treatment of numerous psychiatric disorders, e.g. bipolar disorder, eating disorders, posttraumatic stress disorder, alcoholism and schizophrenia (see Arnone, 2005; Johnson *et al.*, 2007). The pharmacology of topiramate is not fully understood, although it has been shown to block AMPA receptors and to potentiate GABA-ergic neurotransmission (White *et al.*, 1997; Arnone, 2005). Clinical studies suggest that treatment with topiramate may improve general psychopathology (Tiihonen *et al.*, 2005) and particularly negative symptoms in schizophrenia (Drapalski *et al.*, 2001; Deutsch *et al.*, 2003) when added to a stable antipsychotic drug treatment. In contrast, adding topiramate to patients receiving clozapine has been found ineffective (Dursun & Deakin, 2001) or even to cause worsening of the symptoms (Millson *et al.*, 2002). As mentioned earlier, common adverse effects of particularly some atypical antipsychotic drugs are weight gain and metabolic side effects (see section 1.9.3: Ganguli, 1999; Allison & Casey, 2001). Several clinical studies show that topiramate can antagonize such side effects (Dursun & Devarajan, 2000; Lessig *et al.*, 2001; Ko *et al.*, 2005; Lin *et al.*, 2005).

As mentioned above the pharmacology of topiramate is somewhat elusive and the underlying mechanisms for its beneficial effect in the treatment of schizophrenia still remain unclear. Against this background, we thus studied the effect of topiramate alone and in combination with either the $D_{2/3}$ receptor antagonist raclopride or clozapine on NMDA-induced responses as well as on electrically evoked EPSPs in pyramidal cells of the medial prefrontal cortex.

Using NMDA-induced stimulation we found that neither raclopride nor topiramate when given alone had any effect on NMDA-induced currents in the medial prefrontal cortex. In contrast, topiramate and raclopride in combination significantly potentiated NMDA receptor-mediated currents, although this potentiation did not reach the size of the effect of clozapine on these currents. The potentiating effect of the combination of topiramate and raclopride could be blocked by the selective D_1 receptor antagonist SCH23390 (see Figure 22), hence the potentiating effect should be dopamine-mediated.

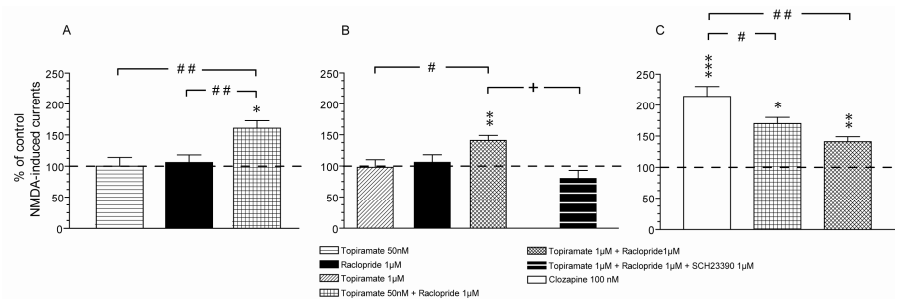

Figure 22. Histograms showing: **A.** the effect of the combination of a low concentration of topiramate and raclopride. **B.** the effect of a high concentration of topiramate and raclopride, on NMDA-induced currents. The effect produced by this combination could be blocked by the D_1 antagonist SCH23390. **C.** The effect of clozapine was significantly higher than the effect of the combination of topiramate and raclopride on the NMDA-induced currents. **P* <0.05, ***P*<0.01, ****P*<0.001 (paired *t*-test), $^{+}$*P*<0.05 (Student's *t*-test), #<0.05, ##<0.01 (one-way ANOVA followed by Tukey HSD test). The holding potential was -60 mV.

We also investigated the electrophysiological effects, in the same model, of the combination of topiramate and clozapine. In these experiments topiramate facilitated the effect of a submaximal concentration of clozapine on NMDA-induced currents. In contrast, topiramate inhibited the potentiating effect of the maximally effective concentration of clozapine on these currents (Figure 23).

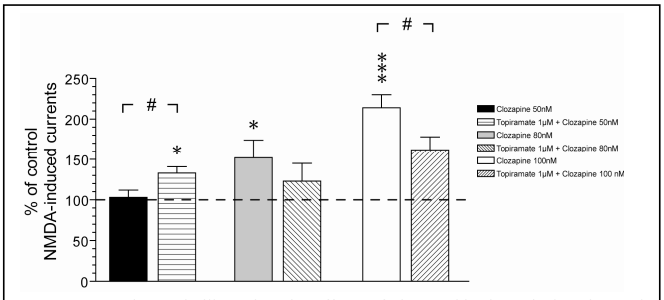

Figure 23. A bargraph illustrating the effects of the combination of clozapine and topiramate on NMDA-induced currens in pyramidal cells of the medial prefrontal cortex. **P* <0.05, ***P*<0.01 ****P*<0.001 (paired *t*-test), #*P*<0.05 (Student's *t*-test). The holding potential was -60 mV.

The mechanism underlying the facilitating effect of the combination of topiramate and raclopride on the NMDA-induced currents may involve an increased dopamine output in the prefrontal cortex (Eltayb *et al.*, 2005), which in turn should lead to dopamine D_1 receptor activation. Since the $GABA_A$ receptor antagonist bicuculline was included in the Ringer's solution throughout the experiment, the pharmacological property of topiramate to facilitate

GABAergic neurotransmission is not likely to contribute to the observed effect. Instead, we suggest that increased extracellular dopamine outflow in the prefrontal cortex is due to its AMPA antagonistic actions. This notion is supported by recent results showing that local administration of a low dose of the AMPA receptor antagonist CNQX significantly increases dopamine output in the prefrontal cortex (Wu *et al.*, 2002). Interestingly, although neither topiramate nor raclopride increased NMDA receptor-mediated currents when given alone, the combination of the drugs did, demonstrating a synergistic interaction. A similar synergistic interaction between topiramate and raclopride was recently demonstrated on dopamine release in the prefrontal cortex (Eltayb *et al.*, 2005).

We also examined and compared the effects of topiramate, raclopride and clozapine on electrically stimulated EPSPs in pyramidal cells of the medial prefrontal cortex. In confirmation of previous electrophysiological studies (Chen & Yang, 2002; Ninan & Wang, 2003), bath perfusion of clozapine potentiated the late component of the EPSPs. In contrast, topiramate when given alone inhibited the EPSPs, while raclopride had no effect on these responses (see Figure 24). Still, the combination of topiramate and raclopride potentiated the EPSPs in the pyramidal cells in a concentration-dependent manner, and this effect could be blocked by the NMDA receptor antagonist APV. In contrast, a high, but not a low, concentration of topiramate inhibited the effect of clozapine on the EPSPs (see Figure 25).

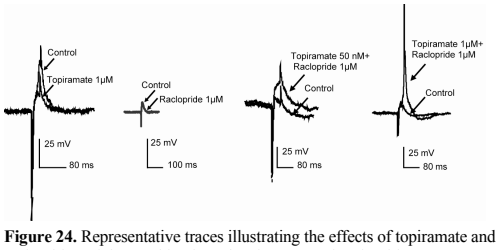

Figure 24. Representative traces illustrating the effects of topiramate and raclopride and the combination of topiramate and raclopride on electrically evoked EPSPs in pyramidal cells of the medial prefrontal cortex.

The concentration of clozapine that elicited the maximum effect on these EPSPs was 100 nM. This concentration corresponds approximately to the plasma concentrations measured in schizophrenic patients treated with clozapine, see section 4.4 (Spina *et al.*, 2000). Consequently, our results may provide an explanation to the clinical observation that topiramate, when used as adjunctive treatment in schizophrenic patients maintained on clozapine, actually may cause a deterioration of symptoms. However, it should be noted that topiramate still was found to potentiate the effect of a lower concentration of clozapine. This observation suggests, in principle, that the addition of topiramate to clozapine might allow for a dose-reduction of clozapine with preserved efficacy concomitant with a reduction of clozapine-related side effects.

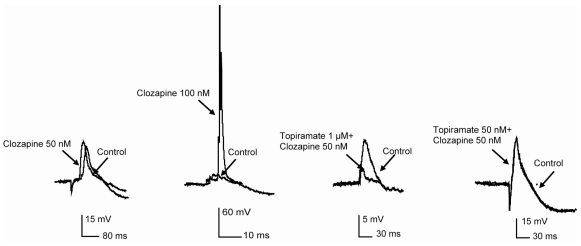

Figure 25. Representative traces illustrating the effects of topiramate and clozapine and the combination of topiramate and clozapine on electrically evoked EPSPs in pyramidal cells of the medial prefrontal cortex.

4.6 Paper IV: Asenapine: antipsychotic activity vs. effects on prefrontal glutamatergic transmission

Asenapine is a novel psychopharmacological agent developed for treatment of schizophrenia and bipolar disorder. Asenapine is a multi-target drug and has higher affinity for an ensemble of serotonergic (5-HT_{2A}, 5-HT_{2C}, 5-HT_6, 5-HT_7), adrenergic (α_{2A}, α_{2B}, α_{2C}), and dopaminergic (D_3, D_4) receptors relative to its D_2 receptor activity. Unlike clozapine, asenapine does not possess differentially higher affinity for histamine H_1 and muscarinic $M_{1\text{-}4}$ receptors relative to D_2 receptors (Andree *et al.*, 1997; Shahid *et al.*, 2007). Emerging clinical trials suggest that asenapine functions as an atypical antipsychotic drug in its ability to improve positive, negative (Potkin *et al.*, 2005) and cognitive symptoms with low EPS liability in schizophrenic patients (Potkin *et al.*, 2007). Some early preclinical studies with asenapine indicated that asenapine might display an antipsychotic effect at relatively high doses (Broekkamp *et al.*, 1990; Costall *et al.*, 1990). However, these previous data do not allow a comparison between asenapine and other, especially the modern antipsychotic drugs in several aspects. Consequently, in the present experiments we employed a broad set of sensitive preclinical methods, which we have previously used to characterize both typical and atypical antipsychotic drugs, to delineate the profile of asenapine which respect to its potential to alleviate not only positive, but also negative and cognitive symptoms in schizophrenia. These included behavioral methods, such as CAR, which measures antipsychotic efficacy with high predictive validity (c.f. above) and the catalepsy test, which allows assessment of EPS liability, but also neurochemical and electrophysiological methods. Thus, microdialysis was employed in awake freely moving animals, to assess the effects of asenapine on regional output of dopamine in brain, and *in vivo* voltammetry was used to study asenapine's effects on dopamine activity in the core and shell of the nucleus accumbens. Finally, using *in vitro* electrophysiology the effect of asenapine on glutamatergic transmission in the medial prefrontal cortex was examined.

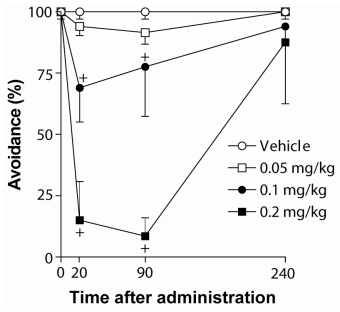

Figure 26. Effects of asenapine on conditioned avoidance response in rats 0 to 240 minutes after drug injection. $^{+}P<0.05$ for saline- vs. asenapine-treated animals (Data analysis and statistics see article IV and section 3.10.2).

We found that acute administration of asenapine produced an effective and selective suppression of CAR at rather low dosage, i.e. about 3 times lower than previously observed (see Figure 26; cf. Broekkamp *et al.*, 1990). This effect was obtained without any associated catalepsy. In fact, the lowest dose of asenapine producing significant catalepsy was 2.5 times higher than the dose needed to produce an antipsychotic-like effect in the CAR test, indicating an atypical profile of asenapine similar to that of risperidone and, olanzapine and to some extent, clozapine, which generates a robust suppression of CAR but induces almost no catalepsy even at very high doses (cf. Wadenberg *et al.*, 1993). Typical antipsychotic drugs, such as haloperidol, produce effective suppression in CAR but also generate catalepsy at rather low doses. Generally, our data thus indicate an atypical antipsychotic profile of asenapine.

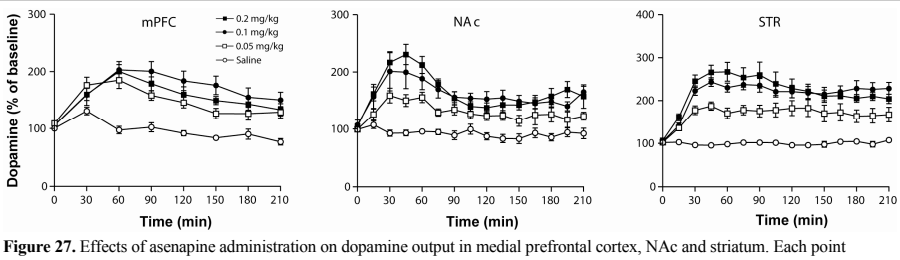

Figure 27. Effects of asenapine administration on dopamine output in medial prefrontal cortex, NAc and striatum. Each point represents the mean ± S.E.M. (Data analysis and statistics see article IV and section 3.10.3).

Our microdialysis studies showed an increased dopamine output in the medial prefrontal cortex (see Figure 27), but also in the striatum and the NAc. As mentioned previously, an increased release of dopamine in the medial prefrontal cortex appears as a commonality among atypical, but not typical, antipsychotic drugs, and this effect is thought to contribute to their enhanced efficacy on negative and cognitive symptoms in schizophrenia. Thus, also these data are consistant with an atypical clinical profile of asenapine.

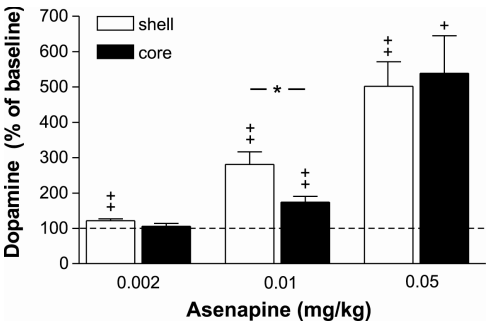

Figure 28. Overall effects of asenapine on extracellular dopamine in the shell and the core of the NAc. Data are presented as mean ± S.E.M. The dotted line represents the baseline value (100%). $^{+}P<0.05$, $^{++}P<0.01$ shell/core vs. baseline, $*P<0.05$ shell vs.core (Data analysis and statistics see article IV and section 3.10.4).

Interestingly, a very low dose of asenapine produced a significant and preferential increase of dopamine in the shell compared to the core of the NAc as measured by *in vivo* voltammetry (see Figure 28). According to previous studies in our laboratory atypical antipsychotic drugs preferentially augment extracellular concentrations of dopamine in the shell of NAc, whereas typical antipsychotic drugs such as haloperidol may preferentially increase extracellular dopamine concentrations in the core region of NAc even at extremely low dosage (Marcus *et al.*, 1996; Marcus *et al.*, 2002). Consequently, these data demonstrate a very substantial difference between e.g. haloperidol and asenapine.

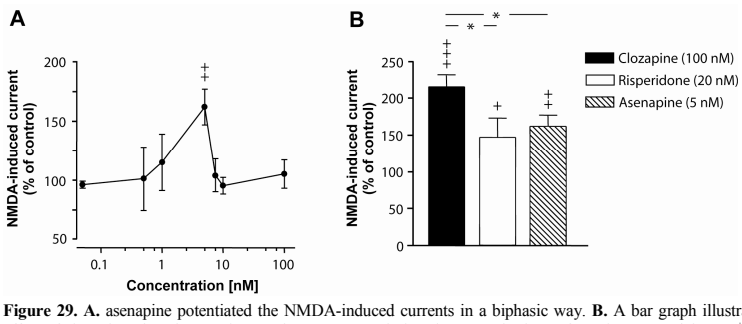

Figure 29. A. asenapine potentiated the NMDA-induced currents in a biphasic way. **B.** A bar graph illustrating the maximal effect of clozapine, risperione and asenapine on NMDA-induced currents in the prefrontal cortecx of the rat. $^{+}P<0.05$, $^{++}P<0.01$, $^{+++}P<0.001$ (paired *t*-test), $*P<0.05$ (Student's *t*-test). The holding potential was -60 mV.

As mentioned earlier, cognitive dysfunction in schizophrenia might be associated with impaired prefrontal dopaminergic as well as glutamatergic transmission. Atypical antipsychotic drugs, e.g. clozapine, but not typical antipsychotic drugs, have as a group been found to facilitate prefrontal glutamatergic transmission (see section 4.2). Our intracellular recordings *in vitro* showed that asenapine potentiated NMDA-induced currents in pyramidal cells of the medial prefrontal cortex in similarity with atypical, but not typical, antipsychotic drugs. However, asenapine potentiated these currents at a very low concentration in the low nanomolar range; i.e much lower than the concentrations needed with clozapine and risperidone. Yet, the maximum effect produced by asenapine was similar to that of risperidone

but lower than that of clozapine (see Figure 29). Our recent preliminary data indicate that asenapine, in similarity with clozapine and olanzapine but not haloperidol (Ninan *et al.*, 2003b), can reverse the PCP-induced functional hyperactivity of NMDA receptors in the pyramidal cells. These results support, in principle, a cognitive enhancing effect of asenapine, a conclusion that seems to be confirmed by recent behavioral data (Neill *et al.*, 2006)

Taken together, our preclinical data propose that asenapine may exhibit potent antipsychotic activity with very low EPS liability. Its ability to increase both dopaminergic and glutamatergic activity in rat medial prefrontal cortex suggest that asenapine may possess an advantageous effect not only on positive symptoms in patients with schizophrenia, but also on negative and cognitive symptoms.

5 SUMMARY AND CONCLUDING REMARKS

In this thesis we demonstrate that both nicotine and topiramate can augment the effect of raclopride on NMDA receptor-mediated glutamatergic transmission, i.e. on NMDA-induced currents and excitatory postsynaptic potentials in pyramidal cells of the rat medial prefrontal cortex. We have also shown that nicotine can enhance the effect of a weak D_4 antagonist, i.e. L-745870, on the prefrontal glutamatergic transmission. The mechanisms underlying the effects of these drug combinations on the NMDA-induced currents may, in analogy with the effect of clozapine on these currents, involve prefrontal dopamine and D_1 receptors. Moreover, the anticonvulsant drug topiramate was found to facilitate the effect of a submaximal concentration of clozapine on the NMDA-induced currents. In contrast, the potentiating effect of a maximal concentration of clozapine on these currents was inhibited by topiramate. Our data also demonstrate that the GlyT-1 inhibitor NFPS augments the effect of both a submaximal and a maximal concentration of risperidone on the NMDA-induced currents. However, NFPS could not further enhance the effect of a submaximal or a maximal concentration of clozapine on these currents. Finally, we have shown that asenapine, a novel psychopharmacological agent, can produce an effective and selective suppression of CAR without inducing catalepsy and also increases dopamine efflux in both the medial prefrontal cortex and the nucleus accumbens. However, in contrast to haloperidol, a very low dose of asenapine produced a preferential increase of dopamine output in the shell compared to the core of the nucleus accumbens and, in similarity with clozapine but at a considerably lower concentration, asenapine was found to facilitate NMDA receptor-mediated glutamatergic transmission in pyramidal cells of the medial prefrontal cortex. The results suggest that the combination of D_2-like receptor antagonists with nACh receptor agonists or AMPA receptor antagonists may be useful in the treatment of negative and cognitive symptoms in schizophrenic patients. Moreover, our results lend further support to the notion that adjunctive use of agents acting on the glycine site on the NMDA receptor may enhance the efficacy of typical and some atypical antipsychotic drugs in these patients. These data further more help to explain the improvement of negative symptoms when topiramate is used as adjunctive therapy in schizophrenic patients receiving typical antipsychotic drugs, but also shed light on the clinically observed deterioration of symptoms when topiramate is added to full dose clozapine treatment in schizophrenia. The fact that asenapine possesses higher affinity for an ensemble serotonergic (5-HT_{2A}, 5-HT_{2C}, 5-HT_6, 5-HT_7), noradrenergic (α_{2A}, α_{2B}, α_{2C}), and dopaminergic (D_3, D_4) receptors relative to its D_2 receptor activity suggests a complex mode of action of this multi-target agent in the present set of preclinical experiments. For example, blockade of 5-HT_{2A} and α_2 receptors may be involved in the facilitating effect of asenapine on the prefrontal NMDA receptor-mediated transmission, since both the selective 5-HT_{2A} receptor antagonist M100907 alone (Ninan *et al.*, 2003a), and a combination of the selective adrenoceptor α_2 antagonist idazoxan and raclopride (Marcus *et al.*, 2005), have been found to produce a similar facilitating effect within this experimental paradigm.

Dysfunction of the prefrontal cortex and cognitive deficits, e.g. in working memory and attention, are common in many psychiatric disorders. Working memory entails the utilization of

an information buffer which serves to guide the appropriate response to a stimulus. Increasing evidence suggests that selective attention plays an important role in working memory. The neuronal representation of the information buffer is sustained by the persistence of neuronal activity of interconnected pyramidal cells in the prefrontal cortex, and activation of this mechanism has been shown to be dependent on both dopaminergic transmission, mediated via the D_1 receptor, and glutamatergic transmission, mediated via the NMDA receptor, in this brain region (see Castner & Williams, 2007). By using electrophysiological techniques we have therefore, in this thesis, investigated the effects of certain drugs and drug combinations on prefrontal NMDA receptor-mediated transmission and excitatory postsynaptic potentials, since facilitation of these potentials is thought to be related to an increased excitability of interconnected pyramidal cells (Chen & Yang, 2002). Consequently, the results from our experiments may correlate with the ability of these drugs and drug combinations to improve some aspects of the cognitive impairment in schizophrenia.

The term "atypicality" basically refers to the initial experience with clozapine, which in contrast to the previously used classical antipsychotic drugs, such as chlorpromazine and haloperidol, was found to exert a potent antipsychotic effect, yet without any EPS; hence it was called an atypical antipsychotic drug. Subsequently clozapine in a classical study (Kane *et al.*, 1988a) was demonstrated to show superior efficacy in otherwise treatment-resistant schizophrenia and, moreover, to provide an advantageous effect on negative symptoms and even cognitive impairment in the patients. By now a range of novel so-called atypical antipsychotics have been developed, including e.g. risperidone, olanzapine and quetiapine to name a few, which as a group has been claimed to cause less EPS than the traditional antipsychotic drugs. Needless to say, this group of compounds differ considerably, not least with regard to their side effects, and none of them is even close to ideal. Many of the "atypicals" are potent 5-HT_2 receptor antagonists, and preclinical studies support a contributory role of this pharmacological property of the drugs for their clinical characteristics, although also some of the typical antipsychotic drugs share this effect. Interestingly, M100907 alone was found to antagonize some of the cognition-impairing effects of the NMDA receptor antagonist MK-801 in mice and rats (Carlsson *et al.*, 1999; Varty *et al.*, 1999) as well as to enhance cortical glutamatergic transmission (Ninan *et al.*, 2003a). However, when tested alone clinically, M100907 still produced an effect mainly on positive symptoms. Thus, the association between upregulated glutamatergic transmission as such and the clinical symptomatology of schizophrenia is far from clear. Since, as mentioned previously, the degree of cognitive impairment in schizophrenia frequently determines treatment-outcome, the search for novel pharmacological strategies to address this issue has led to attempts to develop separate drugs for cognition enhancement in schizophrenia, as illustrated by the American MATRICS initiative. Such efforts have largely focused on drug targets that may directly or indirectly enhance dopaminergic and glutamatergic transmission in cortical areas as well as cortical cholinergic neurotransmission, in particular the nicotinic receptor system.

Taken as a group, the "atypicals" preclinically appears to share a common effect in enhancing prefrontal dopamine outflow, as discussed already; an effect that may be achievable through various pharmacological means and receptors, including e.g. antagonism of 5-$HT_{2A/2C}$ receptors, alone or in combination with D_2 blockage, 5-HT_{1A} agonism, α_2- adrenoceptor blockage, alone or in combination with D_2-blockage as well as activation of nicotinic receptors.

Available evidence indicates that increasing prefrontal dopamine output secondarily activates D_1 receptors and enhances NMDA receptor mediated glutamatergic transmission in this brain region. Other serotonin receptors might also contribute in this regard, since asenapine, which appears as an effective antipsychotic drug with low EPS liability and displays high affinity for a broad range of serotoninreceptors, was found to enhance prefrontal glutamatergic transmission at very low concentrations. Thus, as indicated by preliminary experimental data, this drug may serve as a cognitive enhancing drug even at doses that do not confer any antipsychotic effect. Generally, in order to generate a combination of high antipsychotic efficacy including effects on negative and cognitive symptoms in schizophrenia, yet with low EPS liability, multi-target drugs may seem advantageous. In addition, the current development of various add-on (adjunctive) strategies, to address discrete symptoms or symptoms clusters in schizophrenia may provide novel means to achieve this goal even when using conventional antipsychotic drugs, in particular in order to ameliorate cognitive dysfunction and negative symptoms. However, as regards the latter set of symptoms, a concomitant reduction in D_2 occupancy appears also desirable, since a high degree of D_2 blockage may even worsen such symptoms and, in addition, contribute to reduce compliance. The present set of studies provide, at the experimental level, part of the underlying neurobiological rationales behind some of these add-on strategies to improve the pharmacological treatment of schizophrenia

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