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**Mode of Action of Asenapine  
vs.  
Other Antipsychotic Drugs**

An Experimental Analysis

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*Till min familj*

## ABSTRACT

Antipsychotic drugs (APDs) are used in the treatment of schizophrenia and manic or mixed episodes associated with bipolar I disorder. Generally, the so-called atypical APDs, such as clozapine, may in contrast to typical APDs improve not only positive, but also negative symptoms and some aspects of cognitive impairment associated with schizophrenia. However, as also the atypical APDs may be associated with severe side effects such as weight gain, dyslipidemia, increased prolactin levels and even agranulocytosis, the need for novel and improved pharmacotherapy in this area is considerable. This need is underlined by the fact that in recent years, the beneficial effects of low doses of atypical APDs in the treatment of depressive symptoms in uni- as well as bipolar depression have been increasingly recognized.

The APD risperidone is one of the most commonly prescribed APDs in the world. In similarity with clozapine, risperidone has, however to a smaller extent, affinity for  $\alpha_2$ -adrenoceptors, an effect that otherwise distinguishes clozapine from most other APDs. We therefore examined if additional  $\alpha_2$ -adrenoceptor blockade could enhance the efficacy of risperidone. The antipsychotic-like effect, extrapyramidal side effect (EPS) liability and effects on critical neurotransmitter systems in the brain were studied. Our data propose that the  $\alpha_2$ :D<sub>2</sub> ratio by risperidone is indeed not optimal and that its antipsychotic-like efficiency can be enhanced by adjunctive treatment with an  $\alpha_2$ -adrenoceptor antagonist, e.g. idazoxan, allowing for reduced dosage and a subsequent reduced EPS liability, yet with maintained efficacy.

Asenapine is a recently approved APD with a unique receptor binding profile, developed for the treatment of schizophrenia and bipolar disorder. Clinical studies have shown that asenapine possesses, in addition to its effect on psychosis and mania, a well tolerated side effect profile. Using a range of well established preclinical methods we therefore analyzed experimentally the mode of action of asenapine, demonstrating an antipsychotic-like activity, low EPS liability and an atypical profile as regards its effects on critical neurotransmitter systems in the brain. Notably, asenapine increased prefrontal dopamine release and subsequently, via a D<sub>1</sub> receptor-mediated mechanism, glutamatergic NMDA-induced transmission in the medial prefrontal cortex (mPFC), mechanisms implicated in cognitive functioning. Moreover, the release of noradrenaline and serotonin was also increased in the mPFC, generally indicating antidepressant activity. The profile of asenapine was shown to be similar in several important aspects to other potent atypical APDs, e.g. clozapine, which is the most efficacious APD presently known, but differs from typical APDs, e.g. haloperidol. It was furthermore shown that differential mechanisms are involved in the effects of asenapine on subcortical and cortical dopamine regulation. These data indicate that the dopamine release in the mPFC may largely depend on an intracortical action. Specifically, asenapine in the mPFC was shown to exhibit a pharmacologically significant 5-HT<sub>2A</sub> receptor and  $\alpha_2$ -adrenoceptor antagonistic activity *in vivo*, which may contribute to the enhanced prefrontal monoamine release.

In other experiments, the combination of low doses of asenapine and the SSRI escitalopram was studied, demonstrating an augmentation of monoaminergic effects of escitalopram as well as facilitation of glutamatergic NMDA- and AMPA receptor-mediated transmission in the mPFC. The results may indicate a mode of action similar to that recently proposed to mediate the rapid and potent antidepressant effects of ketamine and scopolamine. Collectively, our results support, in principle, the clinical utility of adjunctive asenapine in treatment-resistant MDD, and indicate a fast onset of action.

## POPULÄRVETENSKAPLIG SAMMANFATTNING

Antipsykotiska läkemedel används främst för behandling av schizofreni och maniska eller blandade episoder av bipolär sjukdom typ I. Generellt har de så kallade atypiska (andra generationens) antipsykotiska läkemedlen, såsom klozapin, till skillnad från typiska (första generationen) antipsykotiska läkemedlen visats förbättra inte bara positiva utan även negativa symptom och delar av de kognitiva funktionsnedsättningar som är vanligen förekommande vid schizofreni. Behandling med atypiska antipsykotiska läkemedel kan dock vara associerad med besvärande biverkningar såsom viktuppgång, förhöjda blodfetter, ökning av prolaktinnivåer och även agranulocytos, vilket gör att behovet av nya och förbättrade behandlingsalternativ inom detta terapiområde är stort. På senare tid har man även sett goda effekter av adjuvant lågdosbehandling med atypiska antipsykotika i behandlingen av depressiva symptom vid både egentlig depression och bipolär sjukdom.

Asenapin är ett nytt antipsykotiskt läkemedel som är godkänt i Europa för behandling av maniska och blandade episoder vid bipolär sjukdom typ I och i USA även för behandling av schizofreni. Asenapine har en multireceptorbindningsprofil med hög affinitet för t.ex. serotonerga 5-HT<sub>2A</sub> samt noradrenerga  $\alpha_2$  receptorer i relation till dopamin D<sub>2</sub> receptorer.

Kortfattat indikerar våra prekliniska resultat att asenapin bör ha en antipsykotisk effekt med låg risk för extrapyramidala biverkningar, vilket konfirmerar dess atypiska profil. I likhet med andra atypiska, men inte typiska, antipsykotiska läkemedel ökar asenapin dopaminfrisättningen markant i prefrontala cortex samt faciliterar glutamaterg transmission i pyramidcellerna, vilket sannolikt bidrar till förbättring av negativa symptom och kognitiva funktionsnedsättningar. Dessutom har reglermekanismerna för den asenapin-inducerade kortikala frisättningen av monoaminer studerats, vilka visat sig delvis bero på en lokal blockad av  $\alpha_2$ -adrenerga och 5-HT<sub>2A</sub> receptorer i mediala prefrontala cortex. Vi har således *inter alia* visat att asenapin ökar tillgängligheten av serotonin och noradrenalin i cortex, vilket kan bidra till dess effekt på depressiva symptom. För att ytterligare studera asenapins effekt vid behandling av depressiva symptom kombinerades asenapin med det välkända antidepressiva läkemedlet escitalopram, vilket visade på en aktivering av frisättningen av monoaminer i prefrontala cortex, liksom faciliteringen av glutamaterg NMDA och AMPA receptormedierad transmission i pyramidceller i prefrontala cortex. Sammantaget kan dessa resultat förklara att asenapin har effekt på både negativa, kognitiva och depressiva symptom samt, i kombination med escitalopram, en verkningsmekanism som liknar den som anses mediera den potenta och snabbt insättande antidepressiva effekten av ketamin och scopolamin.

Risperidon är ett av världens mest använda antipsykotiska läkemedel. Våra nya data talar för att genom ytterligare blockad av  $\alpha_2$ -receptorer kan en dosreducering av risperidon möjliggöras med bevarad effektstorlek men minskad risk för EPS. Däremot sågs ingen förstärkning av den antipsykotiska effekten vid förstärkt 5-HT<sub>2A</sub> receptorblockad.

Sammantaget synes dessa studier ge en fördjupad preklinisk rational för en rad kliniska resultat och observationer gällande ett nytt psykofarmakologiskt läkemedel, asenapine, med potentiell användning vid flera psykiatriska sjukdomar, såsom schizofreni, bipolär sjukdom och egentlig depression.



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- II. FRÅNBERG O, Wiker C, Marcus MM, Konradsson A, Jardemark K, Schilström B, Shahid M, Wong EH, Svensson TH. (2008) Asenapine, a novel psychopharmacologic agent: preclinical evidence for clinical effects in schizophrenia. *Psychopharmacology* 196(3):417-29.
- III. FRÅNBERG O, Marcus MM, Ivanov V, Schilström B, Shahid M, Svensson TH. (2009) Asenapine elevates cortical dopamine, noradrenaline and serotonin release. Evidence for activation of cortical and subcortical dopamine systems by different mechanisms. *Psychopharmacology* 204(2):251-64.
- IV. FRÅNBERG O, Marcus MM, Svensson TH. (2012) Involvement of 5-HT(2A) receptor and  $\alpha(2)$  -adrenoceptor blockade in the asenapine-induced elevation of prefrontal cortical monoamine outflow. *Synapse* 66(7):650-60.
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## LIST OF ABBREVIATIONS

5-HT	5-hydroxytryptamine (serotonin)
AMPA	$\alpha$ -amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid
ANOVA	Analysis of variance
APD	Antipsychotic drug
BBB	Blood-brain barrier
BDNF	Brain-derived neurotrophic factor
CAR	Conditioned avoidance response
CNS	Central nervous system
COMT	Catechol-O-methyltransferase
CS	Conditioned stimuli
DAT	Dopamine transporter
DOPAC	Dihydroxyphenyl acetic acid
DRN	Dorsal raphe nucleus
ECT	Electroconvulsive therapy
EPS	Extrapyramidal side effects
FC	Frontal cortex
GABA	$\gamma$ -amino butyric acid
HPLC	High performance liquid chromatography
HVA	Homovanillic acid
i.p.	Intraperitoneally
i.v.	Intravenously
LC	Locus coeruleus
L-DOPA	L-dihydroxyphenylalanine
MAO	Monoamine oxidase
MDD	Major depressive disorder
MFB	Median forebrain bundle
mPFC	Medial prefrontal cortex
mTOR	Mammalian target of rapamycin
Nac	Nucleus accumbens
NET	Norepinephrine transporter
NMDA	<i>N</i> -methyl-D-aspartate
PCP	Phencyclidine
PET	Positron emission tomography
PFC	Prefrontal cortex
s.c.	Subcutaneously
S.E.M.	Standard error of the mean
SN	Substantia nigra
SSRI	Selective serotonin re-uptake inhibitor
STR	Striatum
TCA	Tricyclic antidepressant drug
TTX	Tetrodotoxin
UCS	Unconditioned stimuli
VMAT	Vesicular monoamine transporter
VTA	Ventral tegmental area

# 1 INTRODUCTION

## 1.1 Antipsychotic drugs

The first antipsychotic drug (APD) chlorpromazine, developed in the early 1950s, was discovered in the search for novel antihistaminic drugs but was soon found to be effective on psychotic patients (Delay et al., 1952; Laborit and Huguenard, 1951). It was thereby groundbreaking and replaced the at the time available treatment options, which for example included general sedation, insulin coma and surgical lobotomy (Ban, 2001; Black, 1982). Chlorpromazine has a multireceptor binding profile with high affinity for e.g. dopaminergic and histaminergic receptors. Soon after, haloperidol was synthesized, possessing high D<sub>2</sub> receptor affinity and a potent antipsychotic effect (Janssen et al., 1958; Leucht et al., 2009). Drugs such as chlorpromazine and haloperidol were entitled neuroleptics and are now also called typical or first generation APDs.

The above mentioned clinical observations stimulated basic scientists to explore the potential mechanism of action involved. In the 1950s, Carlsson and associates first showed that dopamine is a neurotransmitter in its own right and not only a precursor to noradrenaline (Carlsson et al., 1957, 1958). Soon after, they also discovered that APDs increased dopamine and noradrenaline metabolism, suggesting a compensatory activation due to catecholamine receptor blockage (Carlsson and Lindqvist, 1963). The antipsychotic action was subsequently shown to correlate with dopamine D<sub>2</sub> receptor binding in the brain (Creese et al., 1975, 1976; Seeman et al., 1976).

Even if chlorpromazine and haloperidol were associated with remarkable symptom relief, they were found to induce a wide array of side-effects from both the central and the peripheral nervous system (see e.g. Kahn et al., 2008). These include extrapyramidal symptoms (EPS), such as akathisia (inner restlessness), acute dystonia (involuntary sustained muscle contractions), parkinsonism (tremor, hypokinesia and rigidity) and tardive dyskinesia (involuntary movements of e.g. the tongue, lips, face and extremities). EPS were at that time even considered as a therapeutic marker for APDs, i.e. no separation between therapeutic action and side effects was acknowledged (Hippius, 1989). In addition to EPS, increased prolactin levels is a common side effect of many APDs which may lead to amenorrhea and galactorrhea in women, gynecomastia in men and sexual dysfunction in both genders. Since these issues often are reason for noncompliance or termination of medication by patients, reduction of side effects is of importance to avoid relapses.

Clozapine is the prototype for atypical APD and a chemical analogue to tricyclic antidepressant drugs (TCAs), such as imipramine, although with a potent antipsychotic effect, yet with very low EPS liability (Davis et al., 2003; Gross and Langner, 1966; Leucht et al., 2009). The atypicality of clozapine refers to the prior definition of APDs, where the antipsychotic effect was closely linked to EPS. Although clozapine has a very low EPS liability, it may generate substantial weight gain, sedation as well as agranulocytosis (Idänpään-Heikkilä et al., 1977). The latter observation led to the withdrawal of clozapine from the market, with the exception of some strictly hematologically monitored patients responding well to the treatment. In 1990 clozapine was re-introduced, since studies had shown its effectiveness in otherwise treatment-

resistant patients with schizophrenia (Kane et al., 1988; Lindström, 1988; see also Lewis et al., 2006). In addition, clozapine has been shown to substantially reduce the risk of suicide (see Hennen and Baldessarini, 2005; Meltzer et al., 2003a) and relief of negative symptoms and some aspects of cognitive impairment that mostly is associated with schizophrenia (Leucht et al., 2009; Meltzer and McGurk, 1999). However, the risk of agranulocytosis still reduces its use.

As a common denominator, all presently available APDs share the ability to block dopamine D<sub>2</sub> receptors. Studies using the positron emission tomography (PET) technique have revealed that, whereas most APDs, including haloperidol, requires approximately 70% D<sub>2</sub> receptor occupancy to yield adequate clinical effect, an increased EPS risk emerges at approximately 80%, indicating a rather narrow therapeutic window (Farde et al., 1988), a conclusion that has been supported by subsequent preclinical studies (Wadenberg et al., 2000, 2001b). In contrast, clozapine generates only approximately 45% D<sub>2</sub> receptor occupancy in striatal tissue at clinically effective dosage (Farde and Nordström, 1992), and the low dopamine D<sub>2</sub> receptor occupancy largely explains its very low EPS liability. Apart from moderate blockage of D<sub>2</sub>-like receptors, the superior clinical efficacy of clozapine has been attributed to its antagonistic action at serotonergic receptors, in particular 5-HT<sub>2A/2C</sub> receptors (Ashby and Wang, 1996; Meltzer et al., 1989, 2003b; Schotte et al., 1996), although most atypical APDs share this effect of clozapine. However, in contrast to other atypical and typical APDs but in similarity with antidepressant drugs such as mianserin and mirtazapine, clozapine is a highly potent  $\alpha_2$ -adrenoceptors antagonist, an effect suggested to largely explain its unique efficacy (de Boer, 1996; Hertel et al., 1999a; Nutt, 1994; Schotte et al., 1996). Moreover, clozapine has been found to act as a partial agonist at D<sub>1</sub> and 5-HT<sub>1A</sub> receptors (Newman-Tancredi et al., 1996; Salmi et al., 1994a), indicating that also other receptors may contribute to its clinical profile (see e.g. Svensson, 2003b). In the search for novel improved psychopharmacological agents, the group of atypical APDs, also denoted second generation APDs, has successively expanded. Thus, several APDs have been developed, some of which with the multireceptor binding profile of clozapine in mind, including a high 5-HT<sub>2A</sub>:D<sub>2</sub> binding affinity ratio, e.g. risperidone, olanzapine, quetiapine, ziprasidone and most recently asenapine (formerly known as Org 5222; Bymaster et al., 1996; Schotte et al., 1996; Shahid et al., 2009).

Preclinical studies have shown that atypical, such as clozapine, but not typical APDs, such as haloperidol, markedly augment prefrontal dopamine release (Kuroki et al., 1999; Moghaddam and Bunney, 1990; Nomikos et al., 1994). In addition, atypical, in contrast to typical, APDs increase the glutamatergic *N*-methyl-D-aspartate (NMDA)-induced currents in pyramidal cells of the medial prefrontal cortex (mPFC; Ninan et al., 2003a). Moreover, prefrontal dopamine may secondarily, via D<sub>1</sub> receptors, regulate glutamatergic NMDA receptor-mediated transmission (Chen et al., 2004; Goldman-Rakic et al., 2000; Ninan and Wang, 2003). Previous studies have shown that prefrontal dopamine regulation is critically involved in cognition, e.g. working memory, and that dysfunctional D<sub>1</sub> receptors may contribute to cognitive impairment in schizophrenia (Castner and Williams, 2007; Sawaguchi and Goldman-Rakic, 1991). In subcortical areas, atypical but not typical APDs augment dopamine release preferentially in the shell compared to core subregion of the nucleus accumbens (NAc; Marcus et al., 1996,

2000), indicating a more prominent mesolimbic than striatal interaction. These findings are supported by studies of regional *c-fos* expression, comparing the effects in the core and shell subregions of typical vs. atypical APDs (Deutch et al., 1992; Robertson and Fibiger, 1992). The purported beneficial effects on negative symptoms and cognitive impairments by atypical APDs (Meltzer and McGurk, 1999; Riedel et al., 2010) have thus been proposed to be related to a preferential mesocorticolimbic selectivity.

The atypical, or sometimes called third generation, APD, aripiprazole differs from the forementioned APDs by being a partial agonist at D<sub>2</sub> receptors, indicating a “stabilizing”, rather than blocking, effect on dopamine transmission in the brain (Keck and McElroy, 2003). In addition, it is a partial agonist at 5-HT<sub>1A</sub> receptors and an antagonist at 5-HT<sub>2A/2C</sub> receptors. As a result aripiprazole generates less D<sub>2</sub> receptor-associated side effects such as EPS and prolactin increase, as well as very little, if any, weight gain (Kane et al., 2002).

Current limitations of APDs, typical as well as atypical, concern foremost lack of treatment efficiency and various side effects (Lieberman et al., 2005), leading to a high degree of discontinuation of the medication. Therefore, the need for improved pharmacological treatment options is substantial. Even though atypical APDs have more favorable tolerability in some aspects, especially concerning a lower risk of EPS, there is still a considerable risk of side effects such as weight gain, sedation, increased prolactin levels and altered glucose as well as lipid metabolism which may lead to diabetes and cardiovascular diseases. However, treatment with APDs compared with no treatment has indeed been shown to be associated with a reduced mortality in patients with schizophrenia (Tiihonen et al., 2009, 2012). In addition, since the degree of cognitive impairment is considered to determine treatment outcome in schizophrenia as well as bipolar disorder and major depressive disorder (MDD; Atre-Vaidya et al., 1998; Green, 1996; Martinez-Arán et al., 2004; McCall and Dunn, 2003) APDs should ideally improve also cognitive deficits.

For all these reasons, novel and improved pharmacological strategies to treat severe mental disorders are highly needed. This goal may be achieved in several ways, including both novel drugs as well as combinations of drugs with complementary modes of action both as regards their neurobiological effects in the brain and effects on various symptom clusters within the respective disease entities.

APDs are primarily used in the treatment of schizophrenia and manic or mixed episodes associated with bipolar I disorder. In addition, APDs are also used in the case of psychosis related to substance abuse and side effects of treatment of Parkinson's disease. In recent years, the beneficial effects of low doses of atypical APDs in the treatment of depressive symptoms in uni- as well as bipolar depression have been increasingly supported (Chen et al., 2011; Croxtall and Scott, 2010; Debattista and Hawkins, 2009; Hardoy and Carta, 2010; Komossa et al., 2010; Montgomery, 2008; Nelson and Papakostas, 2009; Thase, 2002; Tohen et al., 2010). This is not surprising since schizophrenia and mood disorders have partly overlapping symptomatology (psychosis, depressive symptoms and cognitive impairment) and co-morbidities (e.g. substance abuse and anxiety). In addition, the etiology and gene susceptibility (see e.g., Blackwood et al., 2007; Lichtenstein et al., 2009; Murray et al., 2004) as well as certain aspects of dysregulation of monoaminergic transmission (see e.g., Dunlop and

Nemeroff, 2007; Meltzer and Huang, 2008; Schildkraut, 1965; Wong et al., 2010), are also partly shared. Furthermore, adjunct treatment with APDs can improve the efficacy of antidepressant drugs and *vice versa* (see e.g. Debattista and Hawkins, 2009; Silver et al., 2004; Yatham et al., 2005).

## 1.2 Schizophrenia

Schizophrenia is a devastating, poorly understood and insufficiently treated psychiatric disorder, with a life time prevalence of approximately 1% (Carpenter and Buchanan, 1994; Perälä et al., 2007). The onset of schizophrenia often begins in adolescence or early adult life, somewhat earlier in men than women (see e.g. Häfner, 2003), and tends to have a chronic course. The life expectancy of schizophrenic patients is reduced due to increased risk of somatic illnesses such as cardiovascular disease but also to a considerably increased risk of suicide (Casey et al., 2011; Miles, 1977; Palmer et al., 2005; Sim et al., 2006).

### 1.2.1 Symptomatology

The onset of schizophrenia is usually preceded by a prodromal phase, where depressive and negative symptoms are the most common psychiatric problems, which can last for more than a year (see e.g. Häfner et al., 2005). Furthermore, pre-schizophrenic children have been found to manifest cognitive and neuromotor impairments (Jones et al., 1994). The disease can be manifested in different ways and the core symptoms are often divided into the three clusters, namely positive, negative and cognitive symptoms according to the Diagnostic and Statistical Manual of Mental Disorders (DSM-IV; APA, 2000) or the International Classification of Diseases (ICD-10; WHO, 1992). *Positive symptoms*, also referred to as psychotic symptoms, include episodic abnormal experiences and behaviors e.g. bizarre or paranoid delusions, auditory hallucinations and disorganized speech or thoughts. *Negative symptoms* include e.g. inability to experience pleasure (anhedonia), social withdrawal, apathy and poverty of speech (alogia), which can be very disabling. *Cognitive impairment* is manifested by e.g. impaired attention, executive functioning, learning and working memory. While psychotic symptoms are of a phasic character, with symptom intensity fluctuating over time, negative and cognitive symptoms are of a more stable nature, resulting in reduced quality of life, ability to work and social network. Furthermore, schizophrenic patients have an increased risk of psychiatric co-morbidity such as depression, anxiety and substance abuse, which is associated with worse outcome (Buckley et al., 2009; Sim et al., 2006).

### 1.2.2 Etiology and risk factors

Even though the etiology of schizophrenia is multifactorial and remains poorly understood, the underlying pathophysiology most certainly involves both genetic and environmental factors. A high degree of heritability has been shown in schizophrenia using twin, family and adoptions studies indicating a strong genetic involvement (Cannon et al., 2003; Farmer et al., 1987; Lichtenstein et al., 2009). Several susceptibility genes have been associated with schizophrenia, thought to be involved in

neurotransmission and synaptic function, e.g. dysbindin, neuregulin, Disc1 and the Val allele of catechol-O-methyltransferase (COMT; Blackwood et al., 2001; Egan et al., 2001; Harrison and Weinberger, 2005; Weinberger et al., 2001). However, no single gene seems to be responsible, rather a complex interaction between genes and environmental factors seem more likely. Exposure to risk factors early in life when the brain still is vulnerable and immature may lead to developmental disturbances and, subsequently, schizophrenia. Such risk factors include intrauterine infections (Brown et al., 2004; Buka et al., 2008; Mortenssen et al., 2007), obstetric complications (Cannon et al., 2002), migration (Harrison et al., 1988), urban social living (van Os et al., 2004) as well as cannabis abuse (Andréasson et al., 1987). The hypothesis that schizophrenia is caused by impaired neurodevelopment, is also supported by genetic studies as well as anatomical studies of patients with schizophrenia using brain imaging techniques (see Murray et al., 2004). A consistent finding is the frequently reported volume reduction of hippocampus and amygdala in schizophrenic patients (Watson et al., 2012) believed to be related to impaired cognitive and emotional processing, respectively. Other structural neuroanatomical changes in schizophrenia include enlargement of the lateral and third ventricles (Morgan et al., 2007).

### 1.2.3 The dopamine hypothesis of schizophrenia

The pathophysiology of schizophrenia is most probably multifactorial (see above) and the symptoms of schizophrenia are thought to result from the dysfunction of several neurotransmitter systems, among them the dopamine system having received most attention. The classical dopamine hypothesis assumes schizophrenia to be due to a general upregulation of dopamine transmission in the brain (Carlsson, 1978). This notion was originally based on indirect pharmacological evidence demonstrating that all clinically effective APDs block D<sub>2</sub> receptors (see section 1.1). A hyperdopaminergic state was also indirectly supported by the finding that the dopamine precursor L-dihydroxyphenylalanine (L-DOPA) can induce psychotic-like side effects in the treatment of Parkinson's disease and exaggerate positive symptoms in schizophrenia (Angrist et al., 1974; Jenkins and Groh, 1970). In addition, drugs stimulating dopamine release, such as amphetamine, usually worsen psychosis in schizophrenic patients (Snyder, 1973) and can even induce a psychotic-like state in healthy individuals, effects that in turn can be reversed by APDs (Angrist et al., 1974; Brady et al., 1991; Lieberman et al., 1987). However, postmortem studies showing e.g. increased levels of dopamine or D<sub>2</sub> receptors in the brain have been inconclusive since *inter alia* APDs may up-regulate D<sub>2</sub> receptors (for review see Davis et al., 1991; Guillin et al., 2007). More recently, the development of brain imaging techniques has enabled studies in patients, yielding more direct clinical evidence indicating an augmented presynaptic synthesis of dopamine in the striatum (STR; Abi-Dargham et al., 2009; Hietala et al., 1995; Lindström et al., 1999). In addition, an increased striatal dopamine release has been demonstrated in schizophrenic patients, that was found to be associated with psychotic episodes (Abi-Dargham et al., 1998; Breier et al., 1997; Laruelle et al., 1996). These findings support a prominent role of D<sub>2</sub> receptor blockage by APDs for the improvement of positive symptoms. However, even if typical APDs such as haloperidol may improve positive symptoms, the negative and cognitive symptoms of schizophrenia are usually more treatment-resistant and may even be exacerbated

(Carpenter, 1996). Furthermore, amphetamine has been shown to ameliorate certain aspects of negative symptoms associated with schizophrenia (Angrist et al., 1982; Goldberg et al., 1991; van Kammen and Boronow, 1988). Already in the seventies, a reduced blood flow and a hypofunction of the PFC of schizophrenic patients was observed (Andreasen et al., 1992; Berman and Weinberger, 1990; Ingvar and Franzén, 1974) correlating to cognitive deficits and negative symptoms (da Silva Alves et al., 2008; Driesen et al., 2008; Tan et al., 2007). Moreover, low levels of dopamine in the PFC have been proposed to underlie negative symptoms and impaired cognitive functioning, especially regarding working memory, in schizophrenia (Abi-Dargham, 2011; Goldman-Rakic et al., 2004; Karoum et al., 1987). As mentioned previously, the superiority of clozapine in improving negative and cognitive functions in schizophrenia has been associated with the ability to enhance prefrontal dopamine outflow (see section 1.1). These effects are suggested to be mediated via D<sub>1</sub> receptors in the prefrontal cortex (PFC; Goldman-Rakic et al., 2004). Furthermore, the Val allele of the dopamine metabolizing enzyme COMT, leading to increased dopamine catabolism in the PFC, is more common in families afflicted by schizophrenia (Egan et al., 2001; Weinberger et al., 2001). Specifically, this COMT polymorphism predicts cognitive deficits and indicates an impaired prefrontal dopaminergic transmission in patients with schizophrenia. Therefore, a modified dopamine dysregulation hypothesis of schizophrenia has now been generally accepted, where the psychotic symptoms are thought to be associated with hyperactivity or hyperreactivity of the dopamine projection to ventral parts of the STR and, at the same time, cognitive and negative symptoms may be related to a hypofunctioning dopaminergic neurotransmission in the PFC. Consequently, a stabilization of the dopaminergic mesocorticolimbic systems, rather than a general blockage of dopamine transmission, would be preferred (see Svensson, 2003b).

#### 1.2.4 The glutamate hypothesis of schizophrenia

A hypofunction of glutamatergic transmission, e.g. in the PFC, has also been proposed to be part of the pathophysiology of schizophrenia (see e.g. Javitt, 2010). This hypothesis was originally based on the observation that drugs inhibiting glutamatergic neurotransmission via blockage of the *N*-methyl-D-aspartat (NMDA) receptor, such as phencyclidine (PCP), can produce schizophrenia-like symptoms even in healthy individuals (Luby et al., 1959). These drugs were also found to aggravate existing schizophrenic symptoms in patients, including positive and negative as well as cognitive impairments (Javitt and Zukin, 1991; Jentsch and Roth, 1999; Luby et al., 1959).

Preclinical studies have shown that selective NMDA receptor blockage generates severe behavioral and cognitive abnormalities, including impaired working memory (Castner and Williams, 2007; Marcus et al., 2005). In further support of this notion, a mouse model expressing only 5% of normal levels of one of the essential NMDA receptor (NR1) subunits displayed severe behavioral abnormalities, similar to those observed in other animal models of schizophrenia, that could be improved when treated with APDs (Mohn et al., 1999). Clinical data have subsequently shown a significantly reduced phosphorylation of the NR1 subunit in the PFC and hippocampus (Emamian et al., 2004), and postmortem studies have demonstrated



impaired expression of several NMDA receptor subtypes in the PFC of schizophrenic patients as well as in bipolar disorder and MDD (Beneyto and Meador-Woodruff, 2008). Taken together, these data strongly support an involvement of NMDA-receptor dysfunction in the pathophysiology of schizophrenia.

As mentioned previously, prefrontal dopamine, acting via a D<sub>1</sub> receptor-mediated mechanism, is considered important for cognitive functioning. There is also *in vitro* evidence suggesting that stimulation of D<sub>1</sub> receptors in the PFC augments NMDA receptor-mediated transmission (Chen et al., 2004). These mechanisms have been suggested to be critically involved in cognitive functioning (Castner and Williams, 2007; Goldman-Rakic et al., 2004). Indeed, previous preclinical work has shown that atypical APDs markedly enhances cortical NMDA receptor-mediated transmission and also can reverse cognitive impairments induced by NMDA receptor antagonists, an effect mediated via D<sub>1</sub> receptors (Chen and Yang, 2002; Jardemark et al., 2010; Ninan and Wang, 2003; Snigdha et al., 2011).

Furthermore, novel leads indicating a clinical antipsychotic effect of e.g. mGluR2/3 agonists, regulating the glutamatergic transmission in the brain, support the glutamatergic involvement in schizophrenia (Mezler et al., 2010; Patil et al., 2007).

Thus, there may be a hypofunctioning of both dopaminergic and glutamatergic transmission in the mPFC, while at the same time there seems to be a dopaminergic hyperresponsiveness in ventral parts of the STR, leading to psychotic symptoms.

### 1.2.5 Serotonergic transmission in schizophrenia

The involvement of serotonin in the pathophysiology of schizophrenia originally emerged from the finding that the psychotomimetic drug lysergic acid diethylamine (LSD) interacts with serotonergic receptors (Gaddum and Hameed, 1954; Woolley and Shaw, 1957). The mental symptoms generated by LSD may include a psychotic state including even paranoia and formal thought disorder as well as negative symptoms such as social withdrawal (see Breier, 1995; Rinkel et al., 1955). However, the most prominent symptom generated by LSD, visual hallucinations, are rarely present in schizophrenia. LSD was later shown to stimulate 5-HT<sub>2A</sub> receptors. This, in turn, was the fundamental reason why most atypical APDs, in particular risperidone, were designed to exert high 5-HT<sub>2A</sub> receptor blocking properties (see section 1.1). Since atypical, in contrast to typical, APDs may have better effect on negative symptoms and some aspects of cognitive impairment (see section 1.2.7), an involvement of the serotonin system and the 5-HT<sub>2A</sub> receptors in negative and cognitive symptoms have been proposed (see e.g. Breier, 1995; Meltzer et al., 2003b). Originally, adjunct treatment with the 5-HT<sub>2A/2C</sub> receptor antagonist ritanserin was found to augment the effects of typical APDs, such as haloperidol, in schizophrenic patients, in particular with regard to anergia, dysphoria, and negative symptoms, as well as antagonism of parkinsonism (Gelders, 1989; Gelders et al., 1986; Reyntjens et al., 1986). These clinical effects were subsequently paralleled by preclinical studies demonstrating increased prefrontal dopamine availability by adding a 5-HT<sub>2A</sub> receptor blocking agent to D<sub>2</sub> antagonists, such as raclopride and haloperidol, and an

enhanced antipsychotic-like effect (Andersson et al., 1995; Ichikawa et al., 2001b; Westerink et al., 2001). In addition, the dopamine and noradrenaline release in the mPFC by clozapine has been shown to be blocked by simultaneous administration of a 5-HT<sub>2A</sub> receptor agonist (Ichikawa et al., 2001a). Although the mechanism is not fully understood, antagonism at the 5-HT<sub>2A</sub> receptor together with D<sub>2</sub> receptor blockade have been found to interact with a 5-HT<sub>1A</sub> receptor-mediated mechanism to enhance cortical catecholamine output (Ichikawa et al., 2001b). Moreover, clinical studies demonstrate augmentation of the effect on negative symptoms using selective serotonin re-uptake inhibitors (SSRIs) as add-on to APDs (Silver, 2004).

### **1.2.6 Noradrenergic transmission in schizophrenia**

Dysfunctional noradrenergic neurotransmitter systems may also contribute to the symptoms of schizophrenia (Yamamoto and Hornykiewicz, 2004). The atypical APD clozapine, as well as asenapine and risperidone (although to a lower extent), have all affinity for  $\alpha_2$ -adrenoceptors (Ashby and Wang, 1996; Schotte et al., 1996; Shahid et al., 2009). This receptor binding profile has been shown to be importantly involved in their clinical action (see section 1.1 and 1.2.7). Indeed,  $\alpha_2$ -adrenoceptors are known to regulate the release of several monoamines, i.e. dopamine, noradrenaline and serotonin, and, in addition, the firing activity of these cells (Devoto et al., 2004; Gobert et al., 1998; Hertel et al., 1999b; Svensson et al., 1975; Wang et al., 2011; see also section 1.5.4). Clinically,  $\alpha_2$ -adrenoceptor receptor antagonists have been shown to enhance the effect of typical APDs in treatment-resistant schizophrenia (Hecht and Landy, 2012; Litman et al., 1996). These clinical findings are supported by preclinical studies demonstrating that additional blockade of  $\alpha_2$ -adrenoceptors enhances the antipsychotic-like effect of low doses of raclopride, a selective D<sub>2</sub> receptor antagonist, as well as APDs with low  $\alpha_2$ -adrenoceptor affinity, i.e. haloperidol and olanzapine, and at the same time increases prefrontal dopamine release and stimulates cortical NMDA receptor-mediated transmission via D<sub>1</sub> receptor activation (Hertel et al., 1999a; Marcus et al., 2005; Wadenberg et al., 2007).

### **1.2.7 APDs in the pharmacotherapy of schizophrenia**

Treatment of schizophrenia is primarily focused on suppression of symptoms and prevention of relapse as well as functional rehabilitation. The therapy is primarily based on pharmacological treatment with APDs but includes also psychosocial interventions such as cognitive, family and group therapy. In addition, the management of possible co-morbidity such as depression, somatic diseases and prevention of drug abuse is crucial. Although typical as well as atypical APDs can produce full remission of positive symptoms in schizophrenia (Leucht et al., 2009), a substantial proportion of patients are still treatment-resistant (Kane et al., 1988, 2011). However, atypical APDs, such as clozapine, have shown better effect on negative symptoms as well as some aspects of cognitive impairment, in comparison with typical APDs (Leucht et al., 2009; Meltzer and McGurk, 1999; Riedel et al., 2010), which is important, as improvement of cognitive impairment is critical for treatment outcome (Green, 1996).

Since a substantial part of the schizophrenic patients are also afflicted by depression (Buckley et al., 2009), a common drug combination is co-treatment with antidepressant drugs, such as SSRIs and mirtazapine, added to APDs. Depressive symptoms are a major cause for suicidality in schizophrenia, as well as bipolar disorder and MDD. Interestingly, modest doses of antidepressant drugs have also been found to enhance the efficacy of APDs and may also for this reason be used as adjunctive treatment in schizophrenia (Berk et al., 2001; Grinshpoon et al., 2000; Joffe et al., 2009; Kasckow et al., 2001; Mizuki et al., 1990, 1992; Poyurovsky et al., 1999, 2003; Shiloh et al., 2002; Silver, 1994; Spina et al., 1994; Stenberg et al., 2010; Terevnikov et al., 2011), and, most significantly, may dramatically reduce the risk of suicide (Tiihonen et al., 2012).

Schizophrenia is most often of a chronic character and the neuropsychiatric treatment lifelong. Therefore, to avoid relapse, reduction of side effects is of importance for compliance to the long-term treatment. Even though long-term treatment with APDs is associated with risk of developing severe side effects such as EPS, weight gain, sedation and metabolic side effects (see section 1.1), it is associated with substantially reduced mortality compared with no antipsychotic use (Tiihonen et al., 2009, 2012).

Against the above mentioned findings and considerations, novel pharmacologic treatment options that may reduce also depressive symptoms in schizophrenia are indeed needed.

## **1.3 Bipolar disorder**

Bipolar disorder, also known as manic-depressive disorder, is a severe psychiatric mood disorder with approximately 1-2% afflicted (Merikangas et al., 2007, 2011). The onset is usually in late adolescence or early adult life and the illness tends to have a chronic progress. Men and women are equally affected. The life expectancy is reduced for patients with bipolar disorder due to a higher mortality from both somatic illnesses (twice as high) as well as due to a high risk of suicide (15 and 22 times increased for men and women, respectively; Ösby et al., 2001).

### **1.3.1 Symptomatology**

Bipolar disorder is associated with recurrent episodes of hyperactivity and episodes of depressive symptoms (APA, 2000; WHO, 1992). Between these episodes, a neutral (euthymic) mood reappears. Mixed episodes, in which features of both hyperactivity and depression are present at the same time, may also occur as well as rapid cycling, i.e. four or more episodes per year. In general the depressive symptoms are predominantly expressed (Judd et al., 2002; Post, 2005). According to the symptomatology, bipolar disorder is usually classified as Type 1 (I) or Type 2 (II), where bipolar I disorder includes manic and depressive episodes, whereas bipolar II disorder is less severe with hypomanic episodes and a more prominent depressive feature. Cyclothymia is defined as alternating mood episodes that do not meet criteria for severe depression or mania.

Mania is characterized by e.g. elevated or irritable mood, euphoria, grandiose or delusional ideas, impulsivity, reduced need for sleep, increased talkativeness and

activity, racing thoughts, distractibility and increased sexuality. In the hyperactive episodes, psychotic symptoms may (mania) or may not (hypomania) occur. Bipolar I disorder displays many similarities with schizophrenia, and mania can be indistinguishable from acute schizophrenia (Mamelak, 1978). Hypomania is a state of hyperactivity less severe than mania and not as disabling. Depressive symptoms include e.g. persistent feelings of hopelessness, sadness, anxiety, guilt, disturbance in sleep and appetite, social withdrawal, reduced sexuality, chronic pain and morbid suicidal ideation (see also section 1.4.1). In addition, cognitive impairment, such as impaired executive functioning, verbal learning and memory and attention, is common in bipolar disorder and can be present in all states of the disease with impact on functional outcome (Atre-Vaidya et al., 1998; Martínez-Arán et al., 2004).

### **1.3.2 Etiology and risk factors**

The pathophysiology of bipolar disorder is still not known, but family, twin and adoption studies reveal a high heritability (Kieseppa et al., 2004; Lichtenstein et al., 2009). However, no single gene seems to be responsible, rather a complex interaction of several genes seems likely. Several susceptibility genes have been identified to be associated with bipolar disorder, genes that may also be involved in schizophrenia, indicating an overlap in genetic vulnerability (see e.g. Blackwood et al., 2007; Lichtenstein et al., 2009; Murray et al., 2004).

Non-genetic risk factors include various illicit drugs and early life stressors such as parental loss, in particular maternal loss (Mortensen et al., 2003). In addition, stressful event may induce relapse in bipolar disorder (Ellicott et al., 1990). Notably, sleep deprivation may induce mania in some patients and produce an antidepressant effect in others (Barbini et al., 1998; Wehr et al., 1987).

Studies of structural brain abnormalities in bipolar disorder have yielded inconsistent results, although some indicate enlargement of the third and lateral ventricles (Morgan et al., 2007).

### **1.3.3 Hypotheses of bipolar disorder**

The switch phenomenon in bipolar disorder, which most frequently occurs from depression to hypomania or mania, is poorly understood, but may be triggered by spontaneous (i.e. non-treatment related) or pharmacological interventions with drugs through different mechanisms (for review see Salvatore et al., 2010). Suggested causative biological factors include abnormalities in catecholamine levels, dysregulation of neurotrophic and neuroplastic factors (such as brain-derived neurotrophic factor; BDNF), hypothalamic-pituitary-adrenal (HPA) axis hyperactivity and circadian rhythms.

The pathophysiology of bipolar disorder is less well understood than in schizophrenia or MDD, although part of the symptomatology overlaps between these different disease entities, and no generally accepted animal model for bipolar disorder exists. However, a dysregulation of the mesocorticolimbic system that is involved in control of emotions, cognition and reward, has been proposed.

In support of this notion, D-amphetamine may in healthy individuals induce symptoms such as euphoria, reduced need for sleep and increased alertness closely resembling those of mania (Angrist et al., 1974; Jacob and Silverstone, 1986). Indeed, some results from studies using brain imaging techniques are consistent with the notion of enhanced postsynaptic dopamine responsivity in the STR of patients with bipolar disorder (Anand, 2000). Bipolar depression may on the other hand be related to a hypodopaminergic state, since depressive symptoms may emerge following withdrawal of amphetamine after chronic use (Watson et al., 1972). In addition, dopamine agonists may effectively reverse depressive symptoms (Goldberg et al., 2004; Silverstone, 1984; Zarate et al., 2004). However, administration of L-DOPA and dopamine agonists to patients with bipolar depression may also induce a switch into hypomania (Murphy et al., 1971; Silverstone, 1984). Further support for this notion includes the fact that acute mania is readily treated with APDs with D<sub>2</sub> receptor blocking properties.

The cognitive impairments associated with bipolar disorder may be present in all phases of the disease, including the euthymic state, and are proposed to be associated with a dysregulation of dopamine and, via D<sub>1</sub> receptor-mediated mechanisms, glutamatergic transmission in the PFC (see section 1.2.3 and 1.2.4). The notion that glutamatergic dysfunction may play an important role in the pathophysiology of bipolar disorder has recently been supported by the observation that the NMDA receptor antagonist ketamine can produce a robust and rapid antidepressant effect in treatment-resistant bipolar depression as well as reduce suicidal ideation (Diazgranados et al., 2010a, b; see section 1.4.3). In addition, the NMDA receptor antagonist memantine, which may be used to treat psychosis and cognitive dysfunction associated with Alzheimer's disease (Hellweg et al., 2012; Wilcock et al., 2008), has also demonstrated an anti-manic and mood stabilizing effect in treatment-resistant bipolar disorder (Koukopoulos et al., 2012). Furthermore, both postmortem and genetic studies have proposed altered expression of NMDA as well as  $\alpha$ -amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA) receptors (Beneyto et al., 2007; Beneyto and Meador-Woodruff, 2008; Martucci et al., 2006; Mundo et al., 2003). Among other known pharmacological triggers are exogenous corticosteroids and certain antidepressant drugs (see Salvatore et al., 2010).

### **1.3.4 APDs in the pharmacotherapy of bipolar disorder**

The treatment of bipolar disorder aims to balance the mood without inducing a switch to the opposite state. The most commonly used drugs for treatment of bipolar disorder are mood stabilizers such as lithium and anticonvulsive drugs, e.g. valproate, lamotrigine and carbamazepine. In similarity with the treatment of psychosis in schizophrenia, acute mania also responds well to APDs. Several atypical APDs, i.e. quetiapine, olanzapine, risperidone, aripiprazole, ziprasidone and most recently asenapine, have been approved for acute treatment, as monotherapy or adjunctive therapy, of manic or mixed episodes associated with bipolar I disorder in adults. APDs are now prescribed to almost 50% of the patients treated for bipolar disorder (Gutierrez-Rojas et al., 2010). Furthermore, SSRIs are often used for acute bipolar depression although their continued use confers a risk of conversion to mania or rapid cycling. In addition, many patients with bipolar disorder are treatment-resistant and there is frequently a delay in onset of the effect among existing medications (Judd et al.,

2002), particularly during depressive episodes (Nierenberg et al., 2006). A combination of low to moderate doses of atypical APDs, such as olanzapine, and the SSRI fluoxetine have been shown to generate a potent antidepressant effect with a fast onset of action in treatment-resistant depression (Dubé et al., 2007; Shelton et al., 2005). Notably, monotherapy with quetiapine has been approved for the treatment of bipolar disorder, including bipolar depression, and shows in contrast to SSRIs a rapid onset of action (Cruz et al., 2010; Keating and Robinson, 2007), although the underlying mechanism is not, as yet, fully understood.

## **1.4 Major depressive disorder**

Major depressive disorder (MDD), also known as unipolar depression, is a common, severe chronic and often recurrent affective disorder. It afflicts about 16% of the population (Kessler et al., 2003), with twice as high life time prevalence for women than men and higher incidence in late spring or early summer. The time course of the disorder varies from one episode lasting for weeks to a lifelong recurrence of major depressive episodes. The life expectancy for patients with MDD is shorter than normal due to an increased mortality from both somatic illnesses (1.5 times normal) and a high risk of suicide (21 and 27 times for men and women, respectively; Ösby et al., 2001). Over 50% of those committing suicide have affective disorders such as MDD (Cavanagh et al., 2003). Thus, major depression is a severe and disabling disorder associated with very high socioeconomic cost.

### **1.4.1 Symptomatology**

The clinical characteristics of a major depressive episode are similar to those of bipolar depression (see section 1.3.1) and the main symptoms are, according to the DSM-IV, persistent low mood (more than two weeks) and inability to experience pleasure and reward (anhedonia; APA, 2000). Impaired cognition, such as poor concentration, impaired working memory and difficulty making decisions, is often present and its improvement is considered to determine treatment outcome (McCall and Dunn, 2003). In addition, a bias towards negative emotions is typical. In severe cases of MDD psychosis may occur.

### **1.4.2 Etiology and risk factors**

Despite intensive research, the neurobiological basis and pathophysiology of MDD remain largely unknown. Generally, interplay between genes and environmental factors are thought to cause MDD (Chopra et al., 2011; Lohoff, 2010). Heritability may play an important role in the etiology of MDD, as indicated by family, twin, adoption and linkage studies (see Lohoff, 2010). Specifically, polymorphisms in genes regulating the serotonin transporter and BDNF have been shown to correlate with depression (aan het Rot et al., 2009; Lohoff, 2010; McGuffin et al., 2011). Environmental factors such as childhood abuse, ongoing or recent stressful events, reduced ability to cope with stress and psychosocial interactions are also likely to be involved (see e.g. Chopra et al., 2011; Fisher et al., 2012; Popoli et al., 2011; Rosenquist et al., 2011). Furthermore,

inflammation has been proposed to be linked to depression (for recent review, see e.g. Krishnadas and Cavanagh, 2012).

### 1.4.3 Hypotheses of major depressive disorder

Many factors may be involved in the development of MDD, e.g. dysregulation of monoamine transmission, the HPA-axis, neurotrophic factors and cytokines (Chopra et al., 2011). Thus, the monoamine deficiency hypothesis of depression emphasizes the role of monoamines in the pathophysiology and treatment of depression (for review see Carlsson, 1976; Chopra et al., 2011). Despite intense research, direct clinical data supporting a causative role of monoamine-deficiency in depression are sparse and inconclusive as regards the abnormalities in e.g. plasma, serum, CSF and brain tissue and more recently genetic and imaging data. Hence, the monoamine deficiency hypothesis still resides largely on indirect pharmacological evidence. Early studies showed that the depressogenic effect of reserpine in humans very likely was related to depletion of brain monoamines, and, moreover, that the antidepressive effect of monoaminoxidase inhibitors was associated with enhanced monoaminergic transmission (see Schildkraut, 1965). Furthermore, depressive-like symptoms, such as dysphoria, anhedonia, anxiety and insomnia, may emerge following withdrawal from sustained use of high doses of cocaine or amphetamine (Gawin and Kleber, 1986; Watson et al., 1972). Modern pharmacological treatment of depression includes a number of drugs that all enhance monoaminergic transmission in several ways, such as mirtazapine exerting  $\alpha_2$ -adrenoceptor antagonist action, SSRIs, the selective serotonin and noradrenaline re-uptake inhibitor (SNRI) venlafaxin, the noradrenaline re-uptake inhibitor (NRI) reboxetine and the dopamine and NRI (DNRI) bupropion. As mentioned previously, catecholamine projections to the PFC are involved in the control of attention, executive functioning and working memory (Goldman-Rakic et al., 2004; Robbins and Arnsten, 2009). In addition, dopamine is involved in reward, motivation and psychomotor activity which may be altered in depression (see e.g., Dunlop and Nemeroff, 2007). In severe MDD, even psychosis may occur, that may be related to abnormal dopamine reactivity in striatal areas of the brain (see section 1.2.3).

Tryptophan is the precursor of serotonin and tryptophan depletion in the diet has been found to induce relapse in patients who had previously suffered from recurrent episodes of major depression (Smith et al., 1997), indicating a prominent role of serotonin in the pathophysiology of depression. With few exceptions, antidepressant treatments, including SSRIs, TCAs, 5-HT<sub>1A</sub> agonists as well as electroconvulsive therapy (ECT), may as a common denominator exert their effect through modulating the serotonergic system (see Blier et al., 1987; Carlsson et al., 1969; Coppen et al., 1967). However, the therapeutic response to SSRIs, the most commonly prescribed drugs, is often insufficient and usually requires several weeks of treatment before a sufficient antidepressant effect is obtained (Trivedi et al., 2006). The mechanism behind the delayed onset of antidepressant effect may involve neuroadaptation such as desensitization of autoreceptors (Svensson and Usdin, 1978), which corresponds in time with the onset of action of traditional antidepressants in MDD (Blier et al., 1990; Blier and de Montigny, 1987). The antidepressant effect of serotonin has also been proposed to involve activation of 5-HT<sub>2A</sub> receptors on pyramidal cells of layer V in mPFC, thereby enhancing cortical glutamatergic transmission (Aghajanian and Marek,

1997; Li et al., 2010; Miner et al., 2003a; Weisstaub et al., 2006). In fact, an altered expression of the glutamatergic NMDA receptors subunits NR1 and NR2A has been found in MDD (Beneyto and Meador-Woodruff, 2008), indicating a link to cognitive dysfunction. Moreover, NR2B selective NMDA antagonists have been found to produce an antidepressant action in both preclinical depression models and clinical trials (Li et al., 2010; Maeng et al., 2008; Preskorn et al., 2008). Extrasynaptic NR2B receptors have been shown to inhibit the mammalian target of rapamycin (mTOR) signaling and blockage by selective antagonists or the NMDA receptor antagonist ketamine has been demonstrated to re-activate this signaling as well as increase levels of synapse-associated proteins and synaptic spine density in the PFC (Li et al., 2010).

Interestingly, substantial clinical data show that the NMDA receptor antagonist ketamine can improve depressive symptoms after a single injection in treatment-resistant depression with a rapid onset, i.e. within a day compared to weeks for SSRIs, and with a sustained effect, as well as reduce suicidal ideation (Berman et al., 2000; Ibrahim et al., 2012; Price et al., 2009; Zarate et al., 2006; for review see Maeng and Zarate, 2007). Moreover, the ketamine-induced antidepressant effect as well as the activation of the mTOR pathway was blocked by a selective AMPA receptor antagonist (Maeng et al., 2008; Li et al., 2010), indicating a critical involvement of AMPA receptors in the mechanism of action of ketamine. However, due to psychotomimetic and dissociative effects, as well as a potential risk of drug abuse, ketamine has limited clinical utility (Carpenter, 1999; Perry et al., 2007). A disturbed synaptic plasticity seems involved in the pathophysiology of MDD (see e.g. Carlson et al., 2006; Mattson, 2008) and a considerable amount of studies has shown that ketamine and activation of glutamatergic mechanisms as well as stimulation of the mTOR pathway may induce synaptic plasticity (see e.g. Hoeffler and Klann, 2010; Li et al., 2010). Stress and potentially stress-related diseases such as depression may cause atrophy of pyramidal cells in the mPFC, deficits that can be rapidly reversed by ketamine via mTOR-dependent signaling (Li et al., 2010; Liu and Aghajanian, 2008). The reduced hippocampal volume that has been found in MDD (Konarski et al., 2008; Sheline et al., 2003) may potentially involve such mechanisms.

#### **1.4.4 APDs in the pharmacotherapy of major depressive disorder**

The current pharmacotherapy of MDD is based on the principle of enhancing monoaminergic, particular serotonin and noradrenaline, transmission. The most commonly prescribed drugs for the treatment of depression are SSRIs. However, their efficacy is clearly not optimal (Lam and Kennedy, 2004; Trivedi et al., 2006). In addition, the delayed therapeutic response to SSRIs represents a problem as such. In treatment-resistant depression, co-administration of modest doses of atypical APDs to SSRIs or SNRIs has been shown to augment the antidepressant effect with a rapid onset of action, i.e. appearing within days compared to weeks with antidepressant drugs alone (Debatista and Hawkins, 2009; Mahmoud et al., 2007; Nelson and Papakostas, 2009; Nemeroff, 2005; Olver et al., 2008; Tohen et al., 2010), although the mechanisms involved are not fully understood.



## 1.5 The dopamine systems

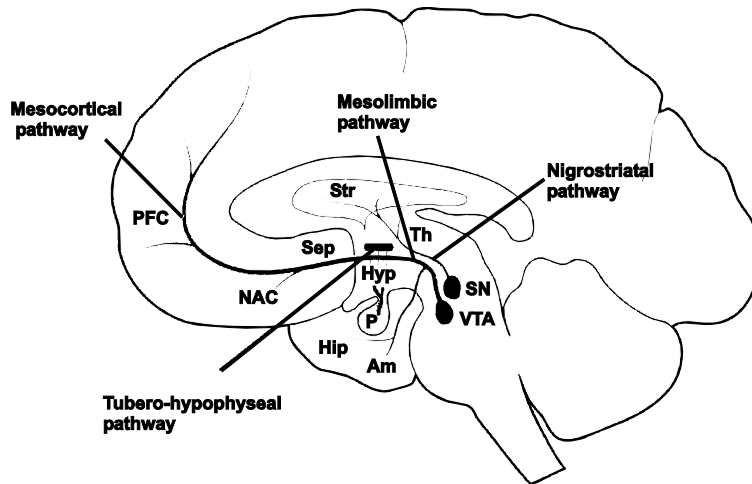
In the 1950s, Carlsson and colleagues discovered that dopamine, i.e. the precursor to noradrenaline and adrenaline, is a transmitter in its own right (Carlsson et al., 1957, 1958; Carlsson, 1959). However, dopamine may also be co-released from noradrenergic neurons (Devoto et al., 2001). The action of dopamine has been described in the terms of gating of inputs and modulation of information, in contrast to having either an inhibitory or excitatory role. Dopamine is now known to be involved in a wide range of behavioral activities such as motor functioning, working memory, reward, mood and stress reactions. The dopaminergic neurotransmission is accordingly a target for drugs used in the treatment of several neurological and psychiatric disorders, including schizophrenia (Carlsson and Lindqvist, 1963; Laruelle et al., 1996), Parkinson's disease (Carlsson, 1959; Hornykiewicz, 1962), drug abuse and affective disorders (see Dunlop and Nemeroff, 2007; Schildkraut, 1965).

### 1.5.1 The dopamine pathways

The introduction of the formaldehyde histofluorescence method (Falck et al., 1962) enabled visualization of the neuronal distribution of monoamine-containing cell. This led to the mapping of the dopaminergic neurons in the brain, demonstrating two main nuclei, i.e. the substantia nigra (SN; A9) and the ventral tegmental area (VTA, A10; Dahlström and Fuxe, 1964; Ungerstedt, 1971). The dopamine system in the central nervous system (CNS) is usually divided into four main pathways; the *nigrostriatal*, the *mesolimbic*, the *mesocortical* and the *tuberoinfundibular* pathway (see Figure 1). A large group of the dopamine neurons are situated in the SN and project mainly to the dorsal STR, i.e. the caudate and the putamen, and constitute the nigrostriatal pathway (Andén et al., 1964), which is involved in sensorimotor control and initiation of movements. Decreased dopamine transmission due to cell death of dopamine neurons in the SN is the main cause of Parkinson's disease (Hornykiewicz et al., 1962). Blockade of the nigrostriatal dopamine pathway is also associated with EPS, i.e. motor side effects such as parkinsonism, which frequently occur during treatment with typical APDs, but less so with atypical APDs. The dopamine neurons originating in the VTA forms the so-called mesocorticolimbic pathway and are further subdivided into two pathways according to their efferent projections; the mesolimbic and the mesocortical pathway (Moore and Bloom, 1978; Ungerstedt, 1971). The mesolimbic pathway innervates subcortical areas, e.g. NAc in the ventral STR, amygdala and hippocampus, whereas the mesocortical pathway innervates e.g. the PFC (see section 1.9 and 1.10). Finally, the tuberoinfundibular pathway contains neurons with short axons projecting from the arcuate nucleus of the hypothalamus to the median eminence and pituitary gland via the infundibulum. Dopamine is the predominant inhibitory factor in the synthesis and secretion of prolactin (Fitzgerald and Dinan, 2008). Treatment with D<sub>2</sub> blocking drugs such as APDs may therefore cause increased prolactin secretion and associated side effects (see section 1.1).

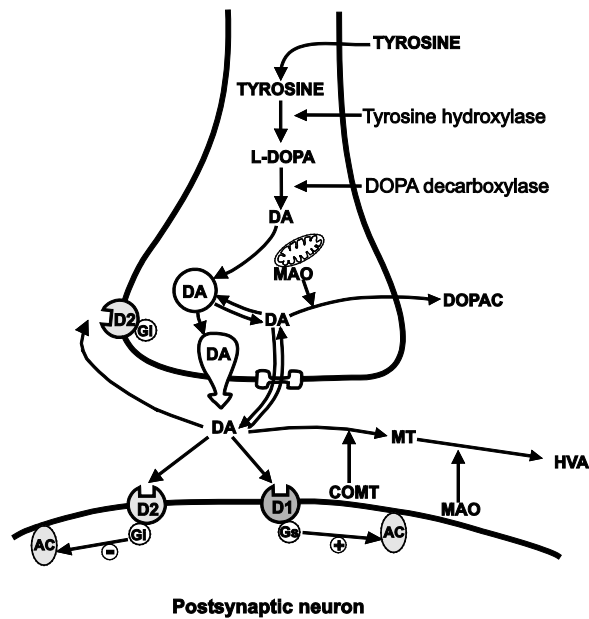
**Figure 1.** Schematic drawing of the dopamine pathways in the human brain.

Abbreviations: Am = amygdaloid nucleus; Hip = hippocampus; Hyp = hypothalamus; NAc = nucleus accumbens; P = pituitary gland; PFC = prefrontal cortex; Sep = septum; SN = substantia nigra; STR = striatum; Th = thalamus; VTA = ventral tegmental area. Modified from (Rang et al., 1999).



### 1.5.2 Biosynthesis and elimination of dopamine

Dopamine, as all catecholamines, is formed from the non-essential amino acid tyrosine (see Hardman et al., 2001). L-tyrosine is taken up from the blood stream with active transporters over the blood-brain barrier (BBB) and into the catecholamine neurons, including dopaminergic, noradrenergic and adrenergic neurons in the brain. The first enzymatic transformation in the dopamine synthesis is when L-tyrosine is hydroxylated into L-DOPA in a rate-limiting step by tyrosin hydroxylase (see Figure 2). L-DOPA is furthermore decarboxylated into dopamine by DOPA decarboxylase. Dopamine is stored in vesicles in the nerve terminal. This storage can be blocked by drugs such as reserpine, which can deplete neuronal terminals of dopamine, noradrenaline and serotonin by inhibiting the vesicular monoamine transporter (VMAT). Dopamine is released from the catecholamine terminal by a  $\text{Ca}^{2+}$ -dependent exocytic mechanism initiated by a nerve impulse and can also be released dendritically (Nissbrandt et al., 1985). Dopamine can also be co-released from noradrenergic neurons (Devoto et al., 2001, 2003). The primary mechanism for inactivation of dopamine is reuptake into the neurons. Dopamine can be eliminated from the synaptic cleft either into the dopamine neuron by a dopamine selective transporter or into noradrenergic neurons by a norepinephrine transporter (NET; Carboni et al., 1990). These transport proteins can be blocked by inhibitors such as cocaine and various antidepressant drugs. Dopamine may be either recycled or metabolized by the intracellular enzyme monoamine oxidase (MAO) to dihydroxyphenyl acetic acid (DOPAC). Alternatively, extracellular dopamine can be metabolized into 3-methyltyramine (3-MT) and subsequently to homovanillic acid (HVA) by COMT and MAO metabolism. MAO is bound within the surface membrane of the mitochondria and exists in two different forms in the brain, namely MAO-A and MAO-B. While dopamine, noradrenaline and serotonin all are substrates for MAO-A, MAO-B has a preference for dopamine. These enzymes are targets for pharmacotherapies indicated for e.g. depression (MAO-A) and Parkinson's disease (MAO-B). The major metabolite in rat brain is DOPAC, while HVA appears to be predominant in the human brain.



**Figure 2.** 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).

### 1.5.3 Dopamine receptors

The dopamine receptors are G-protein coupled, generating a slow signaling through an intracellular cascade involving second messengers (see Beaulieu and Gainetdinov, 2011; Hardman et al., 2001). The receptors are categorized in two main classes, D<sub>1</sub>-like and D<sub>2</sub>-like receptors, and are classified according to their sequence homology and effect on signaling systems. The D<sub>1</sub>-like receptor subtype includes the D<sub>1</sub> and the D<sub>5</sub> receptors, which activate G<sub>α<sub>s</sub>/olf</sub> proteins, stimulating adenylate cyclase to increase cAMP. The D<sub>2</sub>-like receptors include the long and short isoforms of D<sub>2</sub> (D<sub>2L</sub> and D<sub>2S</sub>) found to be expressed predominantly post- and presynaptic, respectively, as well as the D<sub>3</sub> and D<sub>4</sub> receptors. The D<sub>2</sub>-like receptors are coupled to G<sub>α<sub>i/o</sub></sub> proteins and inhibit adenylate cyclase. The dopamine receptors are diffusely distributed throughout the brain and the expression differs in regional localization in the brain. D<sub>1</sub> receptors are the most expressed dopamine receptor in the brain and found in most areas innervated by dopamine neurons, e.g. in the STR, the NAc, and the frontal cortex (FC). D<sub>5</sub> receptors are poorly expressed in the brain compared to the D<sub>1</sub> receptors. D<sub>2</sub> receptors are expressed in almost the same areas as for D<sub>1</sub> receptors, i.e. in the STR, NAc, and to a significant extent in SN and VTA, however only to a low extent in the FC. In addition, D<sub>2</sub> receptors are found in the pituitary gland, regulating the release of prolactin. The distribution of D<sub>3</sub> receptors are more restricted and found in highest concentrations in the limbic areas such as the shell of NAc. The D<sub>4</sub> receptors have low expression in the brain and found in e.g. the FC and amygdala. While the D<sub>1</sub> receptors are expressed postsynaptically, the D<sub>2</sub>-like receptors are found both pre- and postsynaptically. D<sub>2</sub> receptors act as autoreceptors when located presynaptically and in somatodendritic regions, and adjust the firing rate, synthesis and release of dopamine by negative feedback.

### 1.5.4 Regulation of dopaminergic transmission in the brain

The dopamine neurons are regulated by both afferent input and negative feedback from autoreceptors. It has been shown that dopamine release in the STR partly is regulated by postsynaptic D<sub>2</sub> receptors via long loop feedback mechanisms, involving

e.g. GABAergic neurons, and dependent on dopaminergic nervous impulse flow, which has been demonstrated in studies using e.g. APDs or D-amphetamine (Andén et al., 1971; Bunney and Aghajanian, 1976a, b, 1978). The activity of dopamine neurons in the VTA and SN is also regulated by feedback mechanisms through D<sub>2</sub> autoreceptors located on the dopaminergic soma and dendrites through an inhibitory potassium-dependent response, resulting in a hyperpolarization of the neuron and reduced firing of the cell (Bunney et al., 1973; Innis and Aghajanian, 1987; Lacey et al., 1987). At the nerve terminal, D<sub>2</sub> autoreceptors regulate the synthesis and release of dopamine (Farnebo and Hamberger, 1971; Imperato and DiChiara, 1988; Kehr et al., 1972; see Beaulieu and Gainetdinov, 2011). However, dopamine neurons innervating the PFC appear to be sparsely regulated by D<sub>2</sub> autoreceptors both as regards synthesis-modulating receptors on the nerve terminals and impulse-regulating somatodendritic receptors (Chiodo et al., 1984). There is also an interaction between the dopamine projections, illustrated by the fact that a reduced mesocortical dopamine output may secondarily cause an increased dopamine output in the NAc (see e.g. Deutch et al., 1990). Thus, the mesocortical and the mesolimbic dopamine projections are differentially regulated.

Furthermore, experimental studies have shown that noradrenaline neurons may regulate dopamine activity by several mechanisms. Thus, noradrenaline was initially found to increase dopamine turnover (Andén and Grabowska, 1976; Hervé et al., 1982). Subsequently, local stimulation of the noradrenergic soma in locus coeruleus (LC) was shown to increase burst firing in dopaminergic neurons of the VTA, an effect found to be mediated via excitatory  $\alpha_1$ -adrenoceptors on their cell bodies (Grenhoff et al., 1993; Grenhoff and Svensson, 1993). Moreover, extracellular dopamine in the cerebral cortex can be co-released with noradrenaline from noradrenergic terminals, a process that is primarily controlled by local, presynaptic  $\alpha_2$ -adrenoceptors (Devoto et al., 2001, 2003, 2004; Hertel et al., 1999b; Masana et al., 2011). Furthermore, as the dopamine-selective transporters in the PFC are sparse, dopamine is mainly cleared via the same transporter as noradrenaline (Miner et al., 2003b; Schroeter et al., 2000; Sesack et al., 1998) and competes for re-uptake from the extracellular space in the PFC (Gu et al., 1994). This competition may thus generate elevated prefrontal dopamine concentrations in association with increased noradrenaline output (Gresch et al., 1995; Moron et al., 2002; Pozzi et al., 1994).

Glutamatergic pyramidal neurons originating in the PFC (Sesack and Pickel, 1992) provide an excitatory input to dopaminergic neurons in the VTA (Gariano and Groves, 1988; Sesack and Carr, 2002; Svensson and Tung, 1989). Furthermore, activation of glutamatergic NMDA receptors on the dopamine neurons in the VTA specifically stimulates burst firing activity (Chergui et al., 1993). The serotonergic 5-HT<sub>1A</sub> and 5-HT<sub>2A</sub> receptors have been found to be highly expressed and co-localized on pyramidal cells of the mPFC (Amargós-Bosch et al., 2004; Jakab and Goldman-Rakic, 1998; Wedzony et al., 2008; Willins et al., 1997) and may indirectly influence dopamine transmission. Additional support for a prefrontal glutamatergic regulation of the dopamine neurons in the VTA includes studies demonstrating that activation of 5-HT<sub>2A</sub> receptors in the mPFC stimulates these dopamine neurons, an effect that can be blocked by NMDA receptor antagonists (Bortolozzi et al., 2005). In contrast,

although the mechanism is not fully understood, antagonism at the 5-HT<sub>2A</sub> receptor together with D<sub>2</sub> receptor blockade enhances cortical dopamine release via a 5-HT<sub>1A</sub> receptor-mediated mechanism (Ichikawa et al., 2001b). The serotonergic neurons originating in the midbrain raphe nuclei innervate both the SN and the VTA, as well as dopaminergic terminal areas such as NAc, STR and mPFC (Azmitia and Segal, 1978; Dray et al., 1976; Hervé et al., 1987; Van Bockstaele et al., 1994). Thus, stimulation of serotonergic afferent input has been found to inhibit the dopamine cell activity in VTA (Kelland et al., 1993).

Finally, input from inhibitory  $\gamma$ -amino butyric acid (GABA) neurons to the dopamine neurons in the VTA is provided both via pathways from other brain areas and interneurons within the VTA (see Kalivas, 1993).

## **1.6 The glutamatergic system**

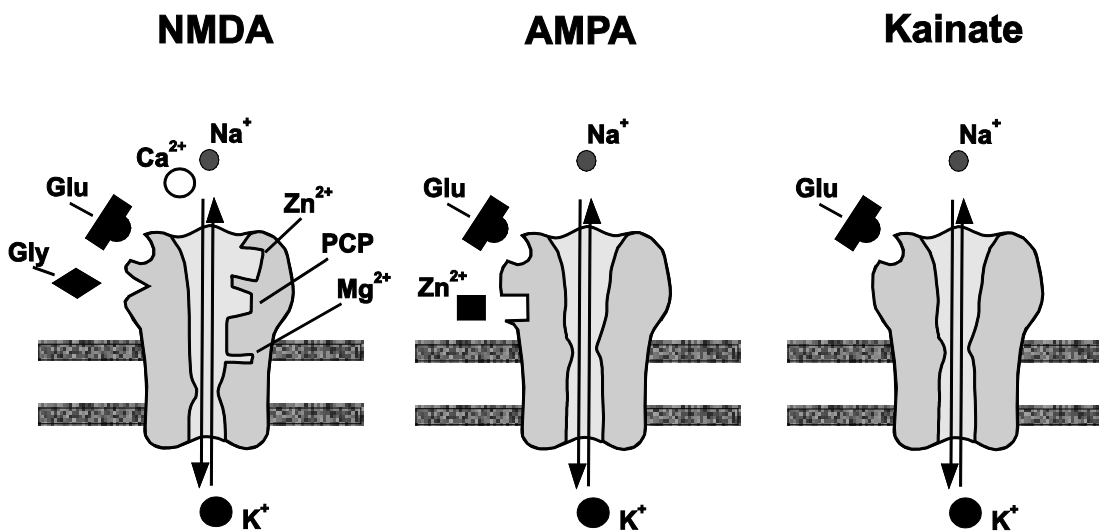
Glutamate is the most abundant excitatory neurotransmitter in the brain and most pathways originate in the cerebral cortex and projects to virtually all regions of the brain (see Hardman et al., 2001). Pyramidal cells in the mPFC and hippocampus are glutamatergic and are involved in synaptic plasticity, cognition, memory and learning (Bortolotto et al., 1999; Castner and Williams, 2007; Collingridge and Bliss, 1995; Li et al., 2010). However, excess of glutamate is known to cause neurotoxicity (Olney, 1994).

### **1.6.1 Biosynthesis and elimination of glutamate**

Glutamate is produced from  $\alpha$ -ketoglutarate, an intermediate derived from glucose in the Krebs cycle (and thus not from glutamic acid, which is a degradation product of amino acids found in high concentrations in the CNS). Glutamate is stored in vesicles in the nerve terminal and released into the synaptic cleft via interaction with soluble N-ethylmaleimide-sensitive factor attachment receptor (SNARE) proteins in a calcium-dependent mechanism when the nerve terminal is depolarized. The primary inactivation mechanism for glutamate is reuptake, either into the glutamatergic cells or into the adjacent astrocytes, which serves to prevent neurotoxic effects caused by to high glutamate levels. In the astrocyte, glutamate is converted to glutamine by the enzyme glutamine syntetas. Subsequently, glutamine is released and transported back to the glutamate nerve terminal, where it is enzymatically phosphorylated by glutaminase into glutamate and re-packed.

### 1.6.2 Glutamatergic receptors

Glutamate activates a number of ionotropic (ligand-gated) and metabotropic (G-protein coupled) receptors. The ionotropic receptors and their respective subunits are classified as NMDA receptors (NR1, NR2A-D, NR3A and B subunits), AMPA receptors (GluR1, GluR2, GluR3, GluR4 subunits) and kainate receptors (GluR5, GluR6, GluR7, KA1, and KA2 subunits), according to their specific pharmacological characteristics (see Figure 3; Cull-Candy et al., 2001; Huettner, 2003; Zarate and Manji, 2008). The receptors are generally widely distributed in the brain.



**Figure 3.** Schematic drawing of the ionic excitatory receptors. Glu=glutamate; Gly=glycine; PCP=phencyclidine. Modified from (Kandel et al., 1991).

Activation of NMDA receptors is restricted and requires binding of both glutamate and the co-agonist glycine. In addition, the ion channel is blocked by extracellular magnesium ions at resting potential, which may be removed by e.g. AMPA receptor-mediated depolarization of the membrane, thereby permitting passage of  $\text{Ca}^{2+}$  through the pore and into the cell. Glutamate binds with high affinity to NR2 subunits of the NMDA receptor (Anson et al., 1998), while glycine binds to the NR1 subunit (Kuryatov et al., 1994). The subunits of the NMDA receptor are assembled as a heteromer, composed of several NR1 subunits together with at least one NR2 subunit. The NR3 subunit may be co-assembled in an NR1/2 complex (Perez-Otano et al., 2001). NR2B subunits are expressed extrasynaptically and may inhibit the mTOR signaling, involved in synaptic plasticity. The properties of the NMDA receptor depend on its constitution of the subunits. Psychotomimetic drugs such as PCP and ketamine bind to the PCP-site inside the ion channel of the NMDA receptor, indicating a major behavioral significance with bearing on mental disorders such as schizophrenia and MDD (see Figure 3; see above).

Both AMPA and kainate receptors are permeable to  $\text{Na}^+$  and  $\text{K}^+$  and mediate a fast excitatory response (Huettner, 2003; Zarate and Manji, 2008). AMPA receptors are often co-expressed with NMDA receptors and its activation results in depolarization of the neuronal membrane. Together they are responsible for the expression and control

(i.e. via  $\text{Ca}^{2+}$  inflow), respectively, of synaptic plasticity. Positive modulators of AMPA receptors may facilitate memory and learning and enhance antipsychotic-like effects of APDs as well as generate an antidepressant effect with fast onset of action in experimental animals (Jardemark et al., 2012; Knapp et al., 2002; Lynch, 1998). Moreover, NR2B selective antagonists and the NMDA receptor antagonist ketamine have been found to generate an antidepressant effect, that is, dependent on AMPA receptor activation in treatment-resistant cases of affective disorder. Thus, a very important role of the glutamatergic system in the neurobiology of depression is indicated (see section 1.3.3 and 1.4.3).

The metabotropic receptors (mGluR) are classified as Group I [mGluR1 (a-d) and mGluR5 (a, b)], Group II (mGluR2/3), and Group III (mGluR4, mGluR6, mGluR7 and mGluR8) according to the specific signaling transduction pathways they activate. Interestingly, as mentioned above, mGluR2/3 receptor agonists seem to possess an antipsychotic effect as judged from initial clinical trials (Mezler et al., 2010; Patil et al., 2007).

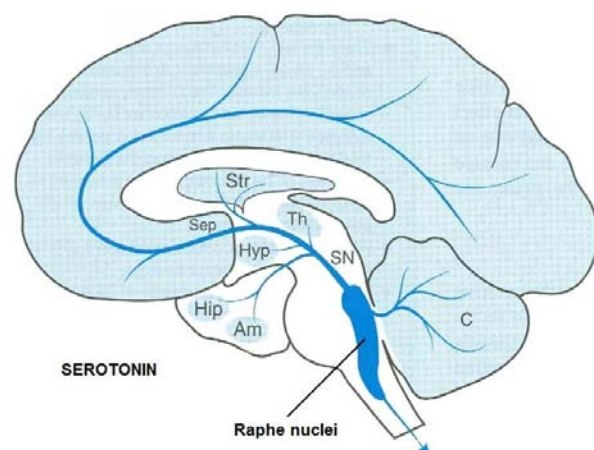
## 1.7 The serotonergic system

Serotonergic neurons project to virtually all parts of the brain (Azmitia and Segal, 1978) and have been implicated in the control of appetite, anxiety, aggression, sexual behavior, mood, pain and perceptual changes such as visual hallucinations (Roth, 1994; Barnes and Sharp, 1999). Serotonin is also considered to be involved in the pathophysiology of several neuropsychiatric diseases such as anxiety disorders, depression and schizophrenia.

### 1.7.1 Serotonin pathways

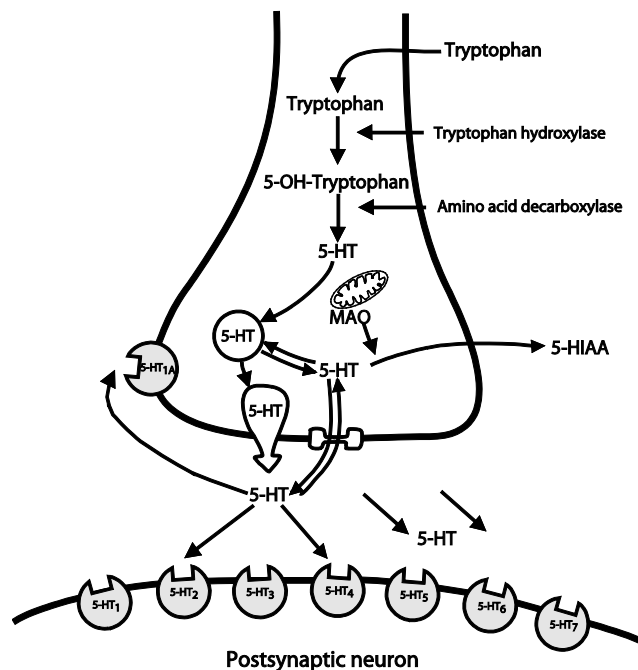
The central serotonergic neurons are found in nine clusters (denoted B1-B9) along the midline of the brain stem, often referred to as the raphe nuclei (see Figure 4). The neurons located in the dorsal (DRN) and median (MRN) raphe project together with noradrenergic neurons via the medial forebrain bundle (MFB) to a large proportion of the brain, e.g. the cortex, hippocampus, limbic systems, thalamus and hypothalamus (Azmitia and Segal, 1978; Rang et al., 2012). Notably, the FC and STR are innervated by the DRN. The caudally located cells project to brain stem structures, the cerebellum and the spinal cord.

**Figure 4.** Serotonergic pathways in the human brain. Am= amygdaloid nucleus; C= cerebellum; Hip= hippocampus; Hyp= hypothalamus; Sep= septum; SN= substantia nigra; Str= striatum, Th= thalamus. Modified from (Rang et al., 1999).



### 1.7.2 Biosynthesis and elimination of serotonin

The basic principles of the biochemistry of serotonin are much alike that of catecholamines (see Hardman et al., 2001). The precursor amino acid L-tryptophan, derived from food protein, passes over the BBB into the serotonergic neurons, where L-tryptophan is metabolized by tryptophan hydroxylase (see Figure 5). This enzymatic metabolism is the major rate-limiting step. However, in contrast to the synthesis of catecholamines, the serotonin synthesis is restricted by the availability of its precursor, i.e. tryptophan, in the CNS (Aghajanian and Asher, 1971), which thus can be reduced by a tryptophan-free diet (Gessa et al., 1974). The resultant precursor 5-hydroxytryptophan (5-HTP) is sub-sequently decarboxylated by aromatic amino acid decarboxylase (AADC) into serotonin, which is the final product in the CNS, except in the pineal gland where serotonin is further transformed into the hormone melatonin. Serotonin is stored in vesicles and released by a  $\text{Ca}^{2+}$ -dependent mechanism initiated by nerve impulses. The major mechanism for elimination of serotonin from the synaptic cleft is re-uptake into the neurons by the serotonin transporter. The termination of the activity of serotonin can also be acquired through sequential enzymatic degradation by MAO-A and aldehyde dehydrogenase into 5-hydroxyindole acetic acid (5-HIAA), which is the primary serotonin metabolite.



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

### 1.7.3 Serotonergic receptors

The serotonin receptors consist of 14 subtypes ( $5\text{-HT}_{1-7}$ ) of which all are G-protein coupled, with the exception of the  $5\text{-HT}_3$  receptor which is a ligand-gated ion channel (Barnes and Sharp, 1999). The  $5\text{-HT}_1$  receptors ( $5\text{-HT}_{1A, B, D, E, F}$ ) are negatively coupled to adenylate cyclase. The  $5\text{-HT}_{1A}$  receptors may function as autoreceptors on the soma and dendrites of the neuron regulating cell firing but are also located postsynaptically in the PFC and the hippocampus. The  $5\text{-HT}_{1B}$  and  $5\text{-HT}_{1D}$  receptors are rather similar, both having an autoreceptor function. They are found in high density in the basal ganglia on nerve terminals but also both pre- and postsynaptically on other neurons.  $5\text{-HT}_{1D}$  receptors are furthermore found in the hippocampus.  $5\text{-HT}_{1E}$  receptors are found in high density in caudate nucleus and putamen and are also detected in the hippocampus and amygdala, however their functional significance is not well characterized. Biochemically, the  $5\text{-HT}_2$  receptors ( $5\text{-HT}_{2A, B, C}$ ) activate phospholipase



C (PLC). PLC cleaves phosphatidylinositol 4,5-bisphosphate (PIP<sub>2</sub>), which in turn causes an increase in inositol triphosphate (IP<sub>3</sub>) and diacylglycerol (DAG). Whereas the 5-HT<sub>2A</sub> and 5-HT<sub>2C</sub> receptors are found centrally, 5-HT<sub>2B</sub> receptors are mainly located in peripheral neurons. The 5-HT<sub>2A</sub> receptors are found with highest density in the FC, primarily distributed postsynaptically on glutamatergic pyramidal neurons of layer V, but also to minor extent on GABAergic interneurons (Jakab and Goldman-Rakic, 1998; Willins et al., 1997). 5-HT<sub>2C</sub> receptors are found in high density in SN and caudate nucleus, but are also detected in cortical and limbic structures. The 5-HT<sub>1A</sub> receptors are highly co-localized with 5-HT<sub>2A</sub> receptors in the mPFC, but are, as mentioned previously, functionally opposing each other (Amargos-Bosch et al., 2004; Wedzony et al., 2008). The 5-HT<sub>2A</sub>, 5-HT<sub>2C</sub> and 5-HT<sub>1A</sub> receptors have been extensively studied since several atypical APDs possess high affinity for these receptors (see e.g. Meltzer et al., 2003b).

5-HT<sub>3</sub> receptors are excitatory ligand-gated ion channels, extensively expressed in the lower brain stem and involved in pain regulation. The 5-HT<sub>4</sub>, 5-HT<sub>6</sub> and 5-HT<sub>7</sub> receptors are excitatory and positively coupled to adenylate cyclase. 5-HT<sub>4</sub> receptors are expressed e.g. in STR and hippocampus. 5-HT<sub>6</sub> receptors are expressed in caudate nucleus, NAc and hippocampus. 5-HT<sub>7</sub> receptors are expressed in thalamus, hypothalamus and hippocampus with generally lower levels in areas such as the cerebral cortex and amygdala. The 5-HT<sub>5</sub> receptor class (5-HT<sub>5A, B</sub>) is the least well known among the serotonin receptors, although found to be negatively coupled to adenylate cyclase.

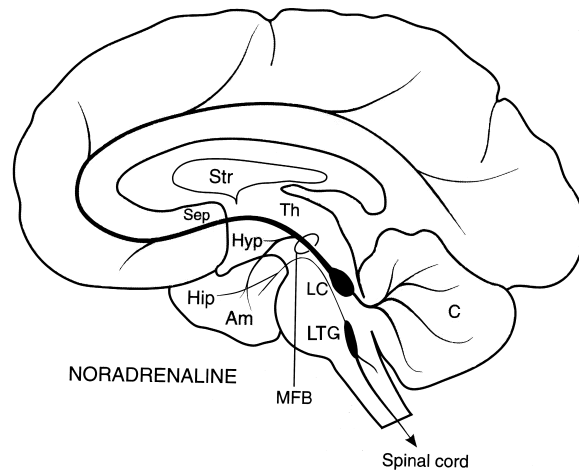
## 1.8 The noradrenergic system

In the 1950s noradrenaline was recognized as a neurotransmitter in the central nervous system (Vogt, 1954). Since then, the noradrenergic system has been shown to be involved in the control of e.g. attention, arousal reactions and exploratory responses to environmental stimuli, especially associated with novelty or fear (Aston-Jones et al., 1999; Svensson and Mathé, 2002) and noradrenaline has also been linked to e.g. mood disorders and schizophrenia (see e.g. Svensson, 2003a; Robbins and Arnsten, 2009; Yamamoto and Hornykiewicz, 2004) and the modes of action of various drugs used to treat these disorders.

### 1.8.1 The noradrenergic pathways

The location and projection of noradrenergic neurons were originally described in the sixties (Andén et al., 1966; Dahlström and Fuxe, 1964; Ungerstedt, 1971). The noradrenergic cell bodies are organized into a number of small clusters in the pons and medulla and are usually divided into two major subgroups; the LC and the lateral tegmental system (see Figure 6). The LC is the most prominent cluster of noradrenergic neurons. The neurons project through the MFB and diffusely innervate large areas of the brain, e.g. the cerebral cortex, limbic system, hypothalamus and cerebellum. The lateral tegmental system shows more diffuse distribution of cells in the medulla and pons. While its ascending pathways mainly projects to amygdala and hypothalamus, the descending pathway projects to the spinal cord.

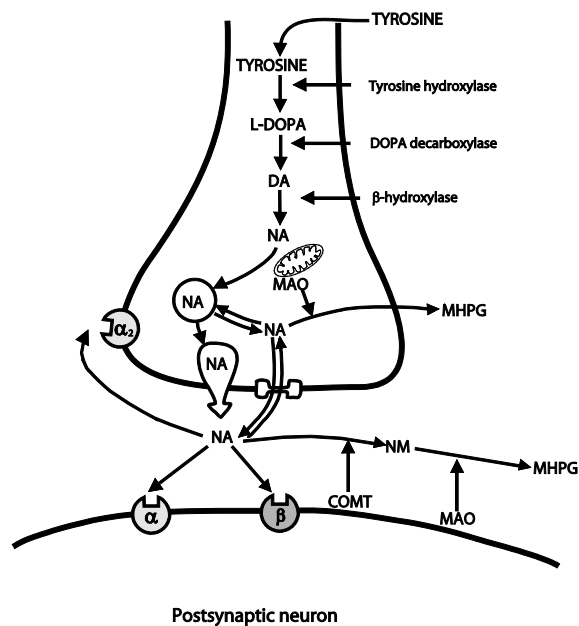
**Figure 6.** Noradrenergic pathways in the human brain. Am= amygdaloid nucleus; C=cerebellum; Hip= hippocampus; Hyp= hypothalamus, LC= locus coeruleus; LTG= lateral tegmental group; MFB= medial forebrain bundle; Sep; septum; Str= striatum; Th= thalamus. Modified from (Rang et al., 1999).



### 1.8.2 Biosynthesis and elimination of noradrenaline

The basic processes for synthesis, storage, release and degradation of noradrenaline are similar to those of dopamine. Synthesis of noradrenaline follows the same route as that of dopamine, however, noradrenergic cells also contain the enzyme dopamine  $\beta$ -hydroxylase, converting dopamine to noradrenaline (see Figure 7; see Hardman et al., 2001). Following its release, noradrenaline is eliminated from the synaptic cleft by a reuptake mechanism involving the NET. Circulating noradrenaline is subject to sequential metabolic degradation by the enzymes COMT and MAO, forming the major CNS metabolite 3-methoxy-4-hydroxy-phenethylglycol (MHPG).

**Figure 7.** Schematic drawing of a noradrenergic neuron.  $\alpha$ =  $\alpha$ -adrenoceptor;  $\beta$ =  $\beta$ -adrenoceptor; NA= noradrenaline; NM= normetanephrine; MHPG= 3-methoxy-4-hydroxy-phenethylglycol. Modified from (Cooper et al., 2003).



### 1.8.3 Noradrenergic receptors

The noradrenaline receptors are G-protein coupled and categorized in three major types, namely  $\alpha_1$ ,  $\alpha_2$ - and  $\beta$ -adrenoceptors. The receptors are further subclassified as  $\alpha_{1A, B, D}$ ,  $\alpha_{2A, B, C}$  and  $\beta_{1, 2, 3}$  (Bylund et al., 1994; Civantos Calzada and Aleixandre de Artiñano, 2001). All of the receptors except for the  $\beta_3$  receptor are expressed in the CNS, and both  $\alpha_1$ - and  $\alpha_2$ -receptors are widely distributed in the brain (Nicholas et al., 1996).  $\alpha_1$  receptors are mostly postsynaptic but may also occur presynaptically and their stimulation activates Gq and subsequently the PLC/IP3 pathway (Bylund et al., 1994).

$\alpha_{2A}$ - and to some extent also  $\alpha_{2C}$ -adrenoceptors are located predominantly presynaptically but may also be found postsynaptically, e.g. in the brain stem (Lee et al., 1998; Nörenberg et al., 1997). At the nerve terminal in the FC,  $\alpha_2$ -adrenoceptors may function both as autoreceptors and heteroreceptors and hereby regulate the release of noradrenaline, dopamine as well as serotonin, and in addition the firing activity of pyramidal neurons (Devoto et al., 2004; Gobert et al., 1998; Hertel et al., 1999b; Svensson et al., 1975; Wang et al., 2011). Stimulation of  $\alpha_2$ -adrenoceptors normally results in a hyperpolarization of the target neuron and inhibition of adenylate cyclase and thus cAMP formation, thereby regulating the release of the transmitter (Limbird, 1988).  $\beta_1$  and  $\beta_2$  receptors are entirely postsynaptic on noradrenaline (or adrenaline) target neurons and glia cells, and their activation results in an increase in cAMP formation by adenylate cyclase via stimulatory G-proteins (see Nicholas et al., 1996; Tate et al., 1991).

## 1.9 The prefrontal cortex

The PFC in the human brain is a region highly involved with the control of cognition and emotions, function modalities that are usually severely affected in major psychiatric disorders such as schizophrenia, bipolar disorder and MDD (Goldman-Rakic et al., 2000; Öngur and Price, 2000; Uylings et al., 2003). Hypofrontality has been especially associated with schizophrenia (Andreasen et al., 1992; Ingvar and Franzén, 1974; Weinberger et al., 1994) and found to be related to certain aspects of cognitive dysfunction as well as negative symptoms (da Silva Alves et al., 2008; Driesen et al., 2008; Tan et al., 2007). There are anatomical and functional similarities of the dorsolateral PFC in humans and primates and the mPFC in the rat (Öngur and Price, 2000; Uylings et al., 2003), although the rat PFC is less well developed and the relative volume much smaller. Four different main areas are included in the mPFC of the rat: the medial agranular, the anterior cingulate, the prelimbic and the infralimbic cortex (Berendse and Groenewegen, 1991; Hoover and Vertes, 2007; Uylings et al., 2003). These areas are heavily connected with each other and to the brain areas projecting to the mPFC, including the thalamus and basal ganglia as well as monoaminergic nuclei such as the VTA, the DRN and the LC (see Hoover and Vertes, 2007; Kuroda et al., 1996; Uylings et al., 2003). Atypical APDs such as clozapine increases dopamine output in the mPFC and secondarily, via  $D_1$  receptors, facilitate

glutamatergic NMDA receptor-mediated transmission in pyramidal neurons of the mPFC, a mechanism highly implicated in cognitive functioning (Castner and Williams, 2007; Chen and Yang, 2002; Goldman-Rakic et al., 2000; Marcus et al., 2005; Ninan et al., 2003a; Sawaguchi and Goldman-Rakic, 1991). The dopamine regulation in the mPFC is rather complex and also dependent on noradrenergic regulation since the DAT is sparse and dopamine inactivation thereby is dependent on re-uptake by the NET (Pozzi et al., 1994; Sesack et al., 1998).

## **1.10 The nucleus accumbens**

The subcortical structure NAc is the main part of the ventral STR and is densely innervated by dopaminergic neurons from the mesolimbic pathway. NAc is generally involved in emotional and motivational processing as well as reward-related functioning. Dysfunction of ventral striatal areas have been shown to be correlated with e.g. psychosis, mania and addiction. In this thesis, the two main subdivisions of the NAc, i.e., the core and shell (Zaborszky et al., 1985), have been further studied. The core region has generally been associated with motor control and the shell region rather with control of drive and emotion (Deutch et al., 1992; Deutch and Cameron, 1992). Atypical APDs, such as clozapine, preferentially increase dopamine output in the shell of NAc, whereas typical APDs, e.g. haloperidol, increase the dopamine output preferentially in the core region (Marcus et al., 1996, 2000, 2002).

## **1.11 Asenapine**

Asenapine is the most recently approved APD for treatment, as monotherapy or adjunctive therapy, of schizophrenia and acute manic or mixed episodes associated with bipolar I disorder in adults in the United States and for treatment of moderate to severe manic episodes associated with bipolar I disorder in adults in Europe (EMA, 2010; FDA, 2009).

The chemical structure of asenapine is tetracyclic and similar to those of the antidepressant drugs mianserin and mirtazapine. Furthermore, asenapine possesses a multireceptor binding profile, with high affinity for several serotonergic (5-HT<sub>1A</sub>, 5-HT<sub>2A</sub>, 5-HT<sub>2B</sub>, 5-HT<sub>2C</sub>, 5-HT<sub>6</sub>, 5-HT<sub>7</sub>), adrenergic ( $\alpha_1$ ,  $\alpha_2$ ) and dopaminergic (D<sub>1</sub>, D<sub>2</sub>, D<sub>3</sub>) receptors with a minimal muscarinic affinity (Schotte et al., 1996; Shahid et al., 2009). Notably, it has higher affinity for e.g. 5-HT<sub>2A/2C</sub>, 5-HT<sub>6</sub>, 5-HT<sub>7</sub> and  $\alpha_{2B}$ -adrenergic receptors than for D<sub>2</sub> receptors (Shahid et al., 2009). Asenapine exerts antagonistic activity at these receptors, with the exception of a partial agonistic action which has been established for the 5-HT<sub>1A</sub> receptor (Ghanbari et al., 2009; Shahid et al., 2009). The receptor binding profile slightly differs from those of e.g. risperidone (5-HT<sub>1A</sub> and 5-HT<sub>6</sub>), olanzapine (5-HT<sub>1A</sub> and  $\alpha_2$ ) and clozapine (muscarinic M<sub>1-4</sub>; Schotte et al., 1996; Shahid et al., 2009). Yet, the basic pharmacology of asenapine at the system level in brain was at the initiation of this study far from understood.

In clinical studies, asenapine has demonstrated potent efficacy on positive symptoms in schizophrenia (Kane et al., 2010; Potkin et al., 2007; Schoemaker et al., 2010, 2012). Its effects on negative symptoms have been shown to be similar to or more efficacious

than those of risperidone and olanzapine (Potkin et al., 2007; Schoemaker et al., 2010, 2012). Several preclinical studies as well as preliminary clinical data indicate an effect of asenapine also on cognitive impairment (Elsworth et al., 2012; Jardemark et al., 2010; Potkin et al., 2006; Snigdha et al., 2011).

In bipolar disorder, asenapine has been shown to be efficacious in the treatment of mixed or manic episodes associated with bipolar I disorder (McIntyre et al., 2009a,b, 2010a, b). Asenapine has also been observed to improve depressive symptoms in bipolar I disorder and schizophrenia as well as in a preclinical model of depression (Kane et al., 2010; Marston et al., 2011; Szegedi et al., 2011). Asenapine is generally well-tolerated with low propensity to induce EPS or anticholinergic effects and has little effect on metabolic parameters and body weight or prolactin secretion (Kane et al., 2010; McIntyre et al., 2009a,b, 2010a, b; Potkin et al., 2007; Schoemaker et al., 2010, 2012).

Against the above background, the present set of preclinical studies were undertaken to reveal the various mechanisms of action of asenapine in brain that might underlie and explain these clinical observations at the systems level, and allow for a more thorough comparison with already well established psychopharmacological drugs with similar clinical utility.

## 2 SPECIFIC AIMS OF THIS STUDY

- To investigate the significance of additional  $\alpha_2$ -adrenoceptor blockade in combination with low doses of risperidone for its antipsychotic-like efficacy, EPS liability, effects on cortical and sub-cortical dopamine efflux and cortical NMDA receptor-mediated transmission in pyramidal neurons in the mPFC.
- To examine the novel APD asenapine as regards its antipsychotic-like effect and EPS liability, and effects on dopamine output in terminal regions of both the nigrostriatal and mesocorticolimbic systems in the brain as well as cortical NMDA receptor-mediated transmission in pyramidal neurons.
- To explore the potentially differential mechanisms of action of asenapine on monoaminergic systems in the mPFC and NAc, with particular focus on dopamine outflow.
- To specifically evaluate the involvement of  $\alpha_2$ -adrenoceptor and 5-HT<sub>2A</sub> receptor blockage in the effects of asenapine on monoamine release in the mPFC *in vivo*.
- To explore if adjunctive treatment with asenapine may enhance the effects of escitalopram on brain neurotransmitters and receptors of critical importance for antidepressant activity.

### 3 MATERIALS AND METHODS

#### 3.1 Animals

Male albino rats were used in all studies. Adult Wistar rats (BK Universal, Sollentuna, Sweden and Charles River Laboratories, Germany) were used for the behavioral, *in vivo* microdialysis and *ex vivo* radioligand binding experiments, whereas Sprague–Dawley rats (BK Universal and Charles River Laboratories) were used for the *in vitro* electrophysiological, *in vivo* electrophysiological and *in vivo* voltammetry experiments.

The animals were housed under standard laboratory conditions in temperature and humidity controlled facilities. Food and tap water were available *ad libitum*. For the microdialysis, voltammetry, electrophysiological and radioligand binding experiments the animals were kept on a 12:12 h light/dark cycle (lights on at 6:00 A.M.), whereas for the behavioral tests, animals were maintained on a reversed 12:12 h light/dark cycle (lights off at 6:00 A.M.). All experiments were performed between 8:00 A.M. and 6:00 P.M. The animals were acclimatized for at least 5 days before experiments. All effort was made to minimize the number of animals used.

In general, all experiments were approved by, and conducted in accordance with, the local Animal Ethics Committee, Stockholm North, and the Karolinska Institutet, Sweden. Permit numbers: N11/00, N339/02, N340/02, N18/04, N93/05, N317/05, N335/05, N338/05, N01/07, N73/08, N387/08, N389/08 and N493/11. However, the radioligand binding experiments were performed at Johnson and Johnson Pharmaceutical Research and Development, Beerse, Belgium and all procedures were approved by the animal care and use committee of Johnson and Johnson Pharmaceutical Research and Development and conducted in strict accordance with the European Communities Council Directive of 24 November 1986 (86/609/EEC).

#### 3.2 Drugs

The following drugs were used:

##### **Antipsychotic drugs**

Asenapine and <sup>3</sup>H-asenapine were gifts from Schering-Plough, Newhouse, UK.

Risperidone was a gift from Johnson & Johnson Pharmaceutical Research & Development, division of Janssen Pharmaceutica NV, Beerse, Belgium.

##### **Antidepressant drugs**

Escitalopram was generously obtained from Lundbeck A/S, Denmark.

##### **Selective noradrenergic drugs**

Idazoxan (an  $\alpha_{2A}$ -adrenoceptor antagonist) and clonidine (an  $\alpha_{2A}$ -adrenoceptor agonist) were purchased from Sigma-Aldrich, St. Louis, USA.

##### **Selective serotonergic drugs**

M100907 [R-(+)- $\alpha$ -(2,3-dimethoxyphenyl)-1-[2-(4-fluorophenyl)ethyl]-4-piperidine methanol; a 5-HT<sub>2A</sub> receptor antagonist] was obtained from Marion Merrell Dow, USA. DOI [1-(2,5-dimethoxy-4-iodophenyl)-2-aminopropane hydrochloride; a 5-HT<sub>2A/2C</sub> receptor agonist] was obtained from RBI, USA.

### Glutamatergic drugs

(RS)-AMPA was purchased from Ascent Scientific, Bristol, UK, and glycine and NMDA were purchased from Sigma-Aldrich, St. Louis, USA.

### Other drugs

Tetrodotoxin (TTX; a voltage-gated Na<sup>+</sup> channel blocker), and bicuculline (a GABA<sub>A</sub> receptor antagonist) were obtained from Tocris, Bristol, UK and Ascent Scientific, Bristol, UK, respectively.

For the *in vivo* microdialysis experiments, drugs were generally dissolved in physiologic saline (0.9% NaCl) for systemic injection. However, risperidone was dissolved in a minimal amount of glacial acetic acid and subsequently diluted in 5.5% glucose for systemic administration. For local application, drugs were generally dissolved in purified water, although escitalopram was dissolved in dimethyl sulfoxine and M100907 in a minimal amount of glacial acetic acid, and then brought to desired stock concentration by addition of purified water. For the local application via reverse microdialysis, the drugs were subsequently diluted in physiologic perfusion solution to reach the final drug concentration. For the *in vitro* electrophysiological experiments, the stock solutions were diluted in Ringer's solution.

Systemic administration was performed either by subcutaneous (s.c.) injections or intraperitoneal (i.p.) injections at a volume of 1.0 ml/kg (behavioral experiments and *in vivo* microdialysis), or by intravenous (i.v.) injections in the jugular vein at a maximum volume of 0.2 ml/kg (*in vivo* voltammetry and *in vivo* extracellular single cell recording). For local administration and recovery experiments (microdialysis), the drugs were infused via the probe at a rate of 2.5 µl /min. All drugs used in the *in vitro* electrophysiological experiments, including NMDA and AMPA, were administered via bath application.

## 3.3 Conditioned avoidance response

### 3.3.1 Procedure

The conditioned avoidance response (CAR) test is a two-way active avoidance response test with a negative reinforcer, proceeded as follows. Rats were trained and tested in conventional shuttle boxes (530×250×225 mm; Kungsbacka Mät- och Reglerteknik, Fjärås, Sweden), divided into two compartments of equal size by a partition with an opening, as described previously (Salmi et al., 1994b). Upon presentation of a conditioned stimulus (CS; an 80 dB white noise), the rats had 10 s to avoid the unconditioned stimulus (UCS; an intermittent electric footshock of 0.3- 0.4 mA [intershock interval 2.5 s, shock duration 0.5 s]), by moving from one compartment of the shuttle box into the other. White noise is produced by combining equal intensity sounds of all different frequencies to form a broad spectrum type of sound. The position of the rat, registered via photocells, was automatically transferred to the computer where the following behavioral variables were recorded (see Table 1): avoidance, escape, escape failure and inter-trial crosses. The animals were trained for five days, and each training session consisted of approximately 20 trials randomly



distributed over 15 min. Only animals reliably performing at a level of >85% avoidance were included in the study. Experiments were preceded by a pre-test and experiment sessions, lasting 10 min, were conducted 20, 90 and 240 min after injection of the drug or vehicle. Experimental days were separated by at least two non-experimental days. The animals were tested in a counterbalanced change-over design serving as their own controls (Li, 1964).

Behavioral variables	Definition
Avoidance	Response to CS within 10 s
Escape	Response to CS + UCS (a maximum of 60 s)
Escape failure	Failure to avoid and escape the UCS by moving to the opposite compartment within 60 s
Inter-trial crosses	Movement between compartments that occur in the absence of either stimulus

**Table 1.** Behavioral variables recorded in the conditioned avoidance response (CAR) test.

### 3.3.2 Methodological consideration

The CAR test has been used since the 1950s and has over time shown high predictive validity for antipsychotic activity (Arnt, 1982; Courvoisier, 1956; Janssen et al., 1965, 1966; Wadenberg, 2010; Wadenberg and Hicks, 1999). In the CAR test, animals exposed to drugs with antipsychotic-like activity exhibit a reduced response to the CS, i.e. avoidance behavior, however moving to the opposite compartment when the UCS is presented, i.e. escape behavior. With a selective suppression of avoidance without induced escape failure, the APDs can be distinguished and separated from e.g. benzodiazepines and barbiturates, which may also suppress avoidance however at doses also affecting escape behavior. Furthermore, the dose needed for APDs to produce antipsychotic-like effect, i.e. approximately 80% suppression of CAR (Wadenberg et al., 2001b), is estimated to produce a striatal D<sub>2</sub> receptor occupancy closely correlating to that needed for sufficient therapeutic response in schizophrenia (Farde et al., 1988; Farde and Nordström, 1992; Wadenberg et al., 2001b; Zipursky et al., 2005), and might thus help to explain the high predictive validity of this test. The mechanisms and structures in the brain involved in the antipsychotic-induced suppression of CAR are still not fully understood. However, local application of a selective D<sub>2</sub> receptor antagonist has demonstrated that the dopaminergic transmission in the nucleus accumbens is involved, i.e. suggesting that the CAR effect is mediated primarily via mesolimbic dopaminergic transmission (Wadenberg et al., 1990). These findings are in line with the notion that psychotic (positive) symptoms are due to a hyperreactivity in the forementioned region (Abi-Dargham et al., 1998; Laruelle et al., 1996).

### 3.4 Catalepsy

#### 3.4.1 Catalepsy procedure

Catalepsy was observed in a dimly lit room by placing the animals on an inclined grid (60°) for a maximum of 2.25 min. The animals were allowed 30 s of adaptation on the grid before observations started.

The catalepsy was scored from 0 to 5, according to the time (square root transformation) the rat remained immobile (see Table 2; Ahlenius and Hillegaart, 1986). If the rat remained immobile for 0.08 min, it was scored as 0, etc. Observations were performed 30,

60, and 120 min after drug or vehicle injection. Each animal received only one treatment.

Catalepsy scores	Time (min)
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 2.** Catalepsy is considered to begin at a score of 2 (see Wadenberg et al. 2001b).

#### 3.4.2 Methodological consideration

The catalepsy test is a robust preclinical test for prediction of EPS liability (Arnt et al., 1981; Wadenberg, 1996). Catalepsy in laboratory animals can be described as a state of motor rigidity which makes the animal incapable to correct an externally imposed unnatural body postures and has been found to correlate well to EPS, a parkinsonian-like motor side effect observed relatively frequently predominantly in patients treated mainly with typical APDs (see section 1.1).

The mechanism of action of catalepsy has been extensively investigated and is suggested to be primarily due to high D<sub>2</sub> receptor blockade in the STR with a consequential imbalance in the dopaminergic and cholinergic systems (Arnt et al., 1981; Fog et al., 1968; Sanberg, 1980). Catalepsy usually appears at about 80% striatal D<sub>2</sub> occupancy (Wadenberg et al., 2000, 2001b), which corresponds well to clinical data where the risk for EPS at this level of D<sub>2</sub> receptor blockade becomes very high in human (Farde et al., 1988; Zipursky et al., 2005). However, some atypical APDs, i.e. clozapine and quetiapine, do not induce catalepsy even in very high dosage (Costall et al., 1978; Wadenberg et al., 2001b).

### 3.5 In vivo microdialysis in freely moving animals

#### 3.5.1 Surgery and microdialysis procedure

A detailed description of the microdialysis technique can be found in all papers included in this thesis. Briefly, rats were anesthesia and mounted into the stereotactic frame. Thereafter, a concentric probe was implanted into the brain area of interest (mPFC, FC, NAc, STR or VTA), in accordance to coordinates in the atlas of Paxinos and Watson (1998). After surgery, the animals were allowed about 48h recovery in the animal facilities before experiments. Dialysis experiments were conducted after connection of the implanted probe to the sampling equipment and performed in awake

and freely moving rats. During experiment, the probe was constantly perfused with physiological perfusion solution, i.e. an artificial cerebrospinal fluid. Using high-performance liquid chromatography (HPLC) coupled to electrochemical detection in a high sensitive analytical cell, separation and quantification, respectively, of the neurotransmitters and their metabolites in the dialysate was accomplished. Administration of the drug was performed after a stable outflow of the neurotransmitters. After completion of dialysis experiment, histological verification of probe placement was performed.

### 3.5.2 Methodological consideration

The microdialysis technique is widely used for detection of extracellular concentrations of substances in tissues, usually *in vivo* (Ungerstedt, 1991). The technique, by means of a small probe equipped with a semipermeable membrane, mimics the function of the blood vessels, allowing substances under a specific diameter to passively diffuse across the membrane along a concentration gradient. It also enables both administration and collection of substances to, as well as from a target area. In addition, samples from awake and freely moving animals can be sampled continuously over a long period of time (hours). However, due to the poor recovery of neurotransmitters over the membrane, the temporal resolution is limited (15 - 30 minutes sampling time depending on brain area studied). Furthermore, since the probe (300  $\mu\text{m}$  in diameter) is rather large the implantation procedure causes local tissue damage and reduces the spatial resolution.

## 3.6 In vitro recovery over the microdialysis membrane

*In vitro* dialysis was performed in a test tube containing a fixed concentration of asenapine with  $^3\text{H}$ -asenapine. A microdialysis probe with an active surface corresponding to the length of mPFC probes was lowered into the solution and perfused with physiological perfusion solution. Samples were collected every 30 minutes and analyzed in a  $\beta$ -counter. The values were compared to a standard curve made by known concentrations of  $^3\text{H}$ -asenapine.

### 3.6.1 Methodological consideration

The employment of local administration of asenapine by reverse dialysis makes the concentrations reaching the brain difficult to assess. Therefore, using a dialysis probe within an *in vitro* setting, the inverse recovery of  $^3\text{H}$ -asenapine passing through the dialysis membrane was investigated. However, the amount of drug reaching the tissue *in vivo* also depends on several other parameters such as the molecular weight of the compound and to the post-implantation gliosis (Nomikos et al., 1990; Robinson and Camp, 1991).

## 3.7 In vivo voltammetry

The *in vivo* voltammetry technique is described in detail in paper II. Briefly, rats were pretreated with the non-selective monoamine oxidase inhibitor pargyline and anesthetized. A tracheal catheter, as well as a jugular and a femoral vein catheter were inserted for i.v. administration of asenapine and continuous infusion of the anesthetic

drug, respectively. The rat was mounted in a stereotaxic instrument and carbon fiber electrodes (active portion 12  $\mu\text{m}$  thick and 500  $\mu\text{m}$  long) were inserted into either the core or the shell region of NAc according to the atlas of Paxinos and Watson (1998). A potential was applied to the electrode, allowing electroactive substances, e.g. catecholamines, in the close proximity of the electrode surface to oxidize and the catechol oxidation current was recorded every minute. After a stable baseline had been achieved, the animals received an injection (i.v.) of saline to exclude nondrug-specific effects, and 10 min later, the drug was injected. At the end of each experiment, an electrolytic lesion was made through the carbon fiber electrode for the following histological verification of the recording site.

### 3.7.1 Methodological consideration

The *in vivo* voltammetry is used to measure neurotransmitter levels in the brain, in similarity with microdialysis (see above). The general principle for the voltammetry technique is based on the observation that monoamines easily can be oxidized and thereby quantified using electrochemical analysis.

In comparison with the aforementioned microdialysis technique, this method has higher temporal resolution, which enables identification of rapid and transient effects. The use of a very fine electrode (12  $\mu\text{m}$  in diameter) enables a higher spatial precision, which allows comparison of small areas of the brain e.g. the core and shell of the NAc. In addition, the injury by the electrode, compared to that of the probe used in the microdialysis, is diminished. In our experimental setting, the animal is required to be anesthetized during the experiment. Although chloral hydrate is considered to interfere minimally with the receptors studied, its influence is not known. However, all animals are equally treated and the results are compared with controls treated in the same way (see also section 3.8.1). 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. Further the pre-treatment of the rats with pargyline prevents formation of DOPAC, thus minimizing the interference of DOPAC with the catechol peak (Gonon, 1988; Gonon et al., 1984). However, the limitation of this method is the incapability to distinguish between dopamine and its metabolite DOPAC or other catecholamines.

In conclusion, there are several differences between the voltammetry and microdialysis techniques which can be used to complement each other when studying pharmacological manipulations of neurotransmitters in the brain.

## 3.8 Extracellular single-cell recording in vivo

The procedures for extracellular single-cell recording are described in detail in Paper III of this thesis. Rats were anesthetized (chloral hydrate) and mounted in a stereotaxic frame. Anesthesia was maintained throughout the experiment. A recording electrode was prepared and lowered into a hole drilled in the skull above the VTA or LC, according to coordinates in the atlas of Paxinos and Watson (1998). Experiments were performed on cells with electrophysiological characteristics corresponding to those previously described for dopamine cells in the VTA and noradrenergic cells in the LC (Graham and Aghajanian, 1971; Wang, 1981). Extracellular electrical activity was amplified, filtered, discriminated and monitored using an oscilloscope and an

audiomonitor. Average firing rate was calculated as spikes per second (Hz). The onset of a burst was defined as an interspike time interval (ISI) of less than 80 ms and burst termination as the next ISI exceeding 160 ms (Grace and Bunney, 1984). Burst firing in VTA dopamine neurons was expressed as the percentage of spikes fired in bursts. Only one cell was studied in each animal.

After completion of the experiment, the recording site was verified histologically. Only animals with correct electrode placement were included in the study.

### 3.8.1 Methodological consideration

The extracellular single-cell recording technique *in vivo* is frequently used to study pharmacological effects on cell firing in different brain areas. In this technique the animal is required to be anesthetized during the experiment. The influence of the anesthetic drug, chloral hydrate, remains unknown and has to be taken into account in the interpretation of the results. However, the results are correlated with control animals treated in the same way (see also section 3.7.1).

## 3.9 Intracellular single-cell recording in vitro

The intracellular single-cell recording technique *in vitro* is described in detail in paper I and II. Using voltage clamp, the voltage is hold constant (clamped), allowing measurement electric currents over the cell membrane, mediated by a specific activation of a receptor/ion-channel complex (e.g. AMPA and NMDA receptors).

### 3.9.1 Preparation of slice

Briefly, the rats were decapitated under anesthesia. The brains were then rapidly removed and cooled in ice-cold Ringer's solution aerated by 95% O<sub>2</sub> / 5% CO<sub>2</sub>. The brains were then cut coronally to produce 450 µm slices. The brain slices were kept submerged in aerated Ringer's solution at room temperature for at least 1 h to allow for recovery. A single slice containing mPFC was then transferred to a recording chamber, for electrophysiological recording. The chamber was continuously perfused by aerated Ringer's solution.

### 3.9.2 Intracellular recording

Intracellular recordings of both AMPA- and NMDA-induced currents in pyramidal cells in layer V and VI of the rat mPFC in slice preparations (approximately anteroposterior +3.2 mm from bregma) were performed in the voltage-clamp mode (see Arvanov et al., 1997; Arvanov and Wang, 1998; Marcus et al., 2012). Electrodes were pulled from borosilicate glass capillaries and filled with 2 M KAc and used for recording the mPFC neurons with an amplifier. Single electrode voltage clamp (holding potential -60 mV) was performed in the discontinuous mode with a sampling rate of 5–6.2 kHz. The voltage recordings are acquired using digital/analogue sampling and acquisition software. Tetrodotoxin (to block the action potentials), glycine (to enhance the NMDA-induced responses) and bicuculline (to block the GABA<sub>A</sub> receptor responses) were routinely included in the aerated Ringer's solution. In order to calculate the effects of the drugs or drug combinations on the prefrontal glutamatergic transmission, the recorded amplitude of NMDA- or AMPA-induced currents after

administration of a drug or a drug combination was divided by the amplitude of the control NMDA- or AMPA-induced current.

The electrophysiological criteria for distinguishing pyramidal from non-pyramidal cells have been described previously (Arvanov et al., 1997; Arvanov and Wang, 1998; Konradsson et al., 2006). In short, the presumed pyramidal neurons of the mPFC have relatively long spike duration (1-3 ms at half maximum spike amplitude) and, in addition, show a pronounced spike-frequency adaptation in response to constant-current depolarization pulses.

### 3.9.3 Methodological consideration

The intracellular single-cell recording technique *in vitro* enables functional studies of the effects of drugs or drug combinations on specific receptor/ion-channels such as AMPA and NMDA. The slice technique enables local application of drugs in a controlled environment, i.e. both drug concentration and ionic composition outside the cells in the slice is controlled.

## 3.10 Ex vivo receptor binding

In this *ex vivo* radioligand binding study, rats were treated by vehicle or risperidone. The animals were decapitated 1 h after drug administration. Brains were immediately removed from the skull and rapidly frozen in dry-ice cooled 2-methylbutane. Sections (20 mm thick) were cut, and thawmounted on microscope slides. The occupancy of  $\alpha_{2A}$ - and  $\alpha_{2C}$ -adrenoceptors by risperidone were measured by *ex vivo* autoradiography using the radioligands [3H]RS79948-197 and [3H]rauwolscine as previously described (Marcus et al., 2005). Quantitative autoradiography analysis was performed with the  $\beta$ -imager according to a standard protocol (Langlois et al., 2001).  $\alpha_{2A}$ - and  $\alpha_{2C}$ -adrenoceptor occupancy were measured by quantifying the specific binding of [3H]RS79948-197 in the septum and the specific binding of [3H]rauwolscine in the STR, respectively. The percentage of receptor occupancy was plotted against dosage and the sigmoidal log dose–effect curve of best fit was calculated by nonlinear regression analysis. From these dose–response curves, the ED50 (the drug dose producing 50% receptor occupancy) were calculated.

## 4 RESULTS AND DISCUSSION

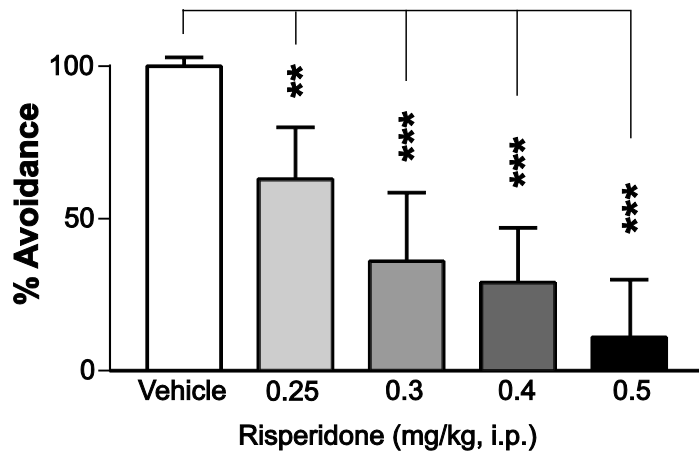
### 4.1 Effects of adjunctive $\alpha_2$ -adrenoceptor or 5-HT<sub>2A</sub> receptor blockade on the antipsychotic efficacy of low doses of risperidone

The superior clinical efficacy of clozapine has, as previously mentioned, been hypothesized to be associated with its potent  $\alpha_2$ -adrenoceptor and 5-HT<sub>2A</sub> receptor blocking properties in combination with a relatively low D<sub>2</sub> receptor occupancy (see Introduction). Both  $\alpha_2$ -adrenoceptor and 5-HT<sub>2A</sub> receptor antagonists have been shown to enhance the effect of typical APDs in treatment-resistant schizophrenia (see Introduction). These clinical results are further supported by preclinical studies demonstrating that adjunctive blockade of  $\alpha_2$ -adrenoceptors enhances the antipsychotic-like effect of low doses of raclopride, a selective D<sub>2</sub> receptor antagonist, as well as APDs with low  $\alpha_2$ -adrenoceptor affinity, i.e. haloperidol and olanzapine, and at the same time selectively increases prefrontal dopamine release (Hertel et al., 1999a; Wadenberg et al., 2007). In addition, an associated facilitation of glutamatergic NMDA receptor-mediated transmission in the mPFC of the rat has been observed when combining an  $\alpha_2$ -adrenoceptor antagonist with a D<sub>2</sub> antagonist (Marcus et al., 2005). Also ritanserin, a 5HT<sub>2A/2C</sub> receptor antagonist, and M100907, a selective 5-HT<sub>2A</sub> receptor antagonist, have been shown to enhance the effect of raclopride and haloperidol, respectively, as regards the antipsychotic-like effect as well as on prefrontal dopamine release (Andersson et al., 1995; Liégeois et al., 2002; Wadenberg et al., 1998, 2001a). In further support of the role of 5-HT<sub>2</sub> receptor blockade, DOI, a 5-HT<sub>2A/2C</sub> receptor agonist, has been shown to attenuate clozapine-induced cortical dopamine release (Ichikawa et al., 2001a).

#### 4.1.1 Additional $\alpha_2$ -adrenoceptor blockage enhances the risperidone-induced antipsychotic-like effect and dopaminergic as well as glutamatergic NMDA receptor-mediated transmission in the mPFC (Paper I)

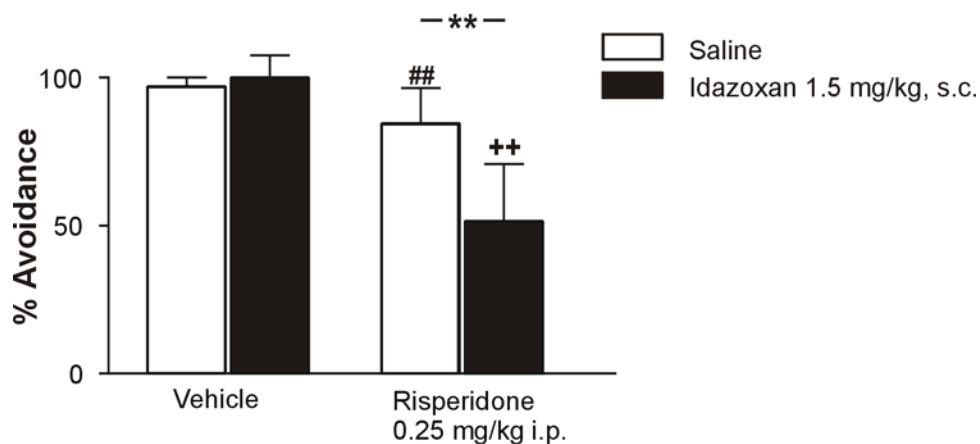
The atypical APD risperidone possesses high 5-HT<sub>2A</sub> receptor affinity but a lower affinity for the  $\alpha_2$ -adrenoceptor than clozapine, although still higher than other atypical APDs, e.g. olanzapine (Schotte et al., 1996; Shahid et al., 2009). We investigated experimentally the ability of adjunctive treatment with the  $\alpha_2$ -adrenoceptor antagonist idazoxan to augment the antipsychotic-like effect of low doses of risperidone using the CAR test. The propensity for causing EPS was also assessed, using a catalepsy test. The effects of this drug combination on dopamine output in the mesocorticolimbic (i.e. mPFC and NAc) terminal regions were measured using *in vivo* microdialysis in awake and freely moving rats. We also investigated the effects of the combination of low concentrations of risperidone and idazoxan on NMDA-induced currents in pyramidal neurons of the mPFC using intracellular electrophysiological recording. Finally, the

$\alpha_{2A}$ - and  $\alpha_{2C}$ -adrenoceptor occupancies of risperidone were assessed using *ex vivo* receptor binding.



**Figure 8.** Effects of risperidone (0.25, 0.3, 0.4, and 0.5 mg/kg i.p.) on CAR behavior in rats at 20 and after administration of drug. The results are presented as median (avoidance %)  $\pm$  semiinterquartile range. Animals are serving as their own control in a change-over design (Li, 1964). \*\*  $p < 0.01$ , \*\*\*  $p < 0.001$  for vehicle vs. risperidone-treated animals.

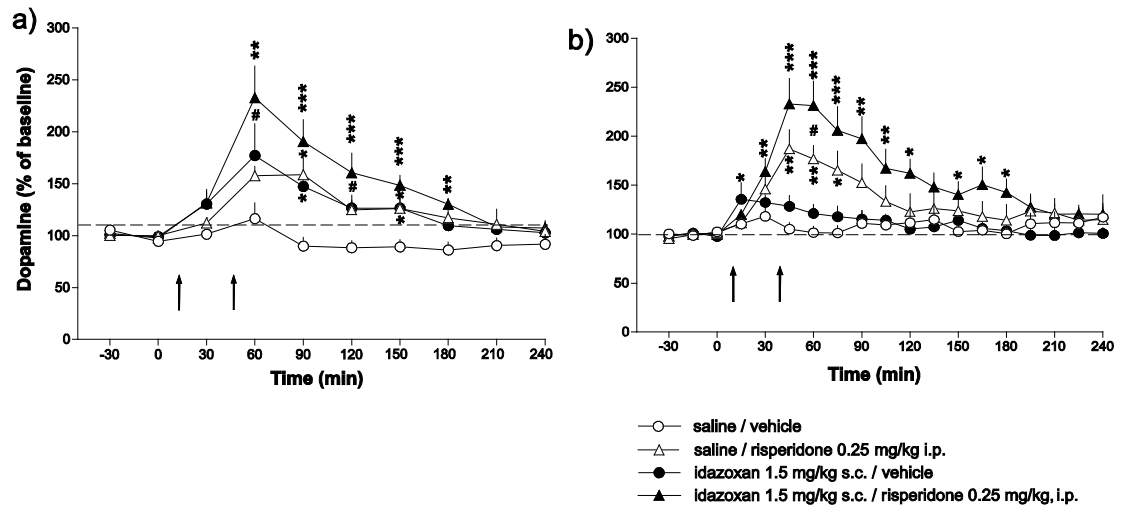
Risperidone induced a dose-dependent suppression of CAR, indicating a potent antipsychotic-like effect (see Figure 8). The dose of risperidone needed for antipsychotic-like effect was between 0.4 and 0.5 mg/kg generating a receptor occupancy of approximately 55-70% at  $D_2$  receptors (Langlois and te Riele, 2008; Wadenberg et al., 2001b) and 12% and 19% at  $\alpha_{2A}$ - and  $\alpha_{2C}$ -adrenoceptor, respectively. Addition of the  $\alpha_2$ -adrenoceptor antagonist idazoxan (1.5 mg/kg) to a reduced dose of risperidone (0.25 mg/kg) enhanced the suppression of CAR, without increasing catalepsy (see Figure 9).



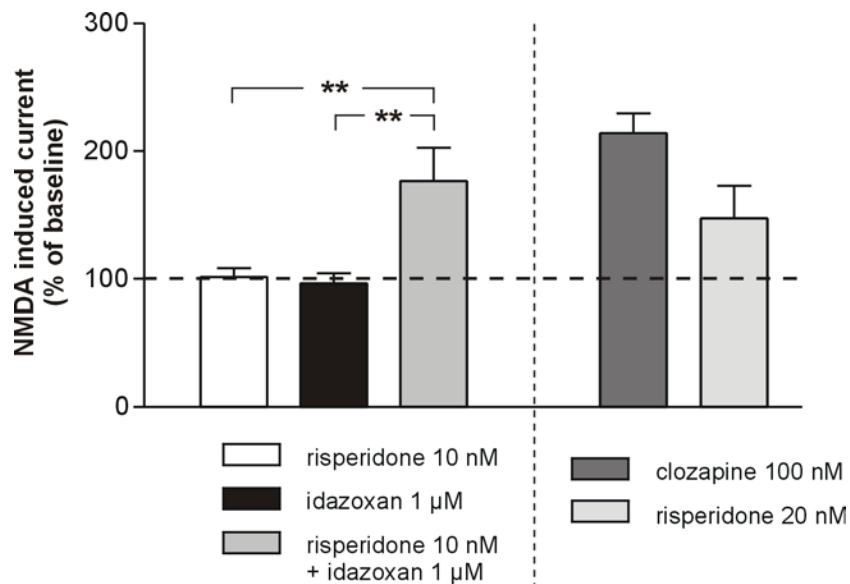
**Figure 9.** Effects of risperidone (0.25 mg/kg i.p.) or idazoxan (1.5 mg/kg s.c.), alone and in combination with risperidone, on CAR behavior in rats at 20 minutes after administration of vehicle or risperidone. The results are presented as median (avoidance %)  $\pm$  semi-interquartile range. Animals are serving as their own control in a change-over design (Li, 1964). ##  $p < 0.01$  risperidone vs. vehicle ; ++  $p < 0.01$  idazoxan vs. the combination of idazoxan and risperidone ; \*\*  $p < 0.01$  risperidone vs. the combination of idazoxan+risperidone.



Both cortical dopamine release and NMDA receptor-mediated responses were enhanced (see Figures 10 and 11).



**Figure 10.** The effects of risperidone (0.25 mg/kg i.p.) administration on dopamine output in (a) the mPFC and (b) the NAc, respectively, in rats pretreated with saline or idazoxan (1.5 mg/kg s.c.). Arrows indicate saline/idazoxan and vehicle/risperidone injections. The results are presented as mean  $\pm$  S.E.M. \*  $p < 0.05$ , \*\*  $p < 0.01$ , \*\*\*  $p < 0.001$  compared to vehicle, #  $p < 0.05$  risperidone vs. idazoxan+risperidone.



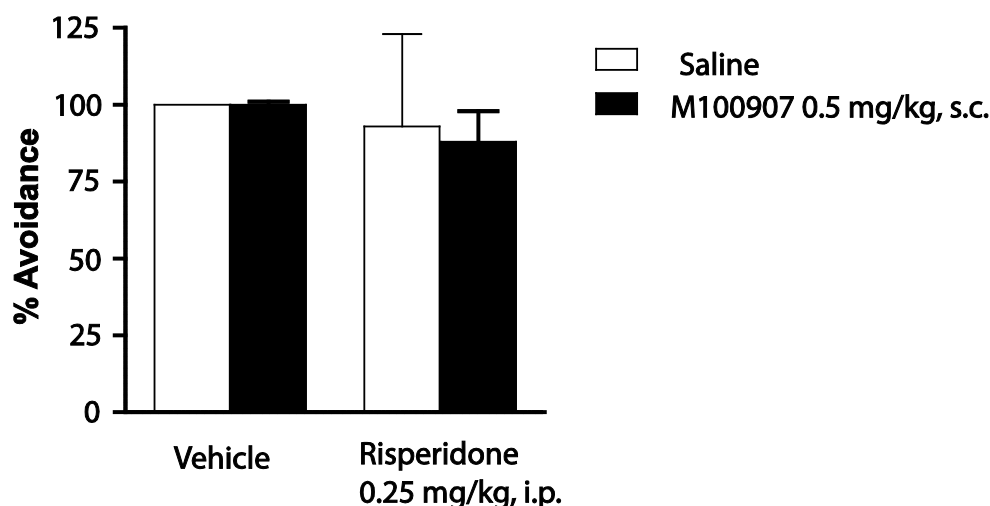
**Figure 11.** The effects of 10 nM risperidone, 1  $\mu$ M idazoxan and the combination of risperidone (10 nM) + idazoxan (1  $\mu$ M) on NMDA-induced currents in pyramidal cells of the mPFC. The results are presented as mean  $\pm$  S.E.M. \*\*  $p < 0.01$  between different treatments. For comparison, the maximal effects of clozapine (100 nM) and risperidone (20 nM; Konradsson et al., 2006) are included in the figure.

The combination of a reduced dose of risperidone (0.25 mg/kg) with idazoxan (1.5 mg/kg) was estimated to generate approximately 50% D<sub>2</sub> receptor occupancy (Wadenberg et al., 2001b) and 75% and 90%  $\alpha_{2A}$ - and  $\alpha_{2C}$ -adrenoceptor occupancy, i.e. similar to that of clozapine (Marcus et al., 2005).

These results propose that the  $\alpha_2$ :D<sub>2</sub> ratio by risperidone is not optimal and that additional  $\alpha_2$ -adrenoceptor blockage may further enhance the antipsychotic effect of low doses of risperidone, yet with a low EPS liability. In addition, the augmented prefrontal dopamine release and facilitation of glutamatergic NMDA receptor-mediated transmission may indicate improvement of both negative symptoms and cognitive impairment.

#### 4.1.2 Additional 5-HT<sub>2A</sub> receptor blockage does not further enhance the antipsychotic-like effect of risperidone (unpublished data)

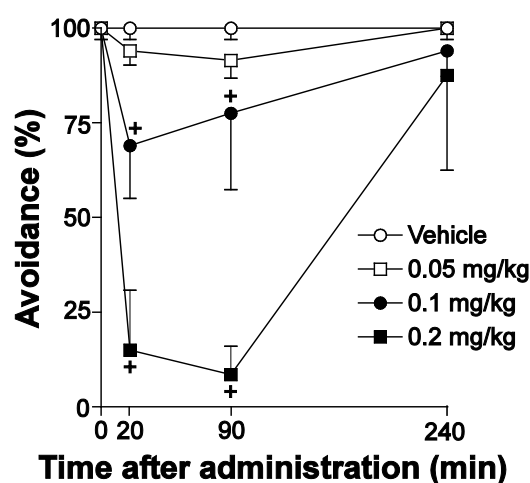
We have further investigated the significance of additional 5-HT<sub>2A</sub> receptor blockade to a low dose of risperidone on the antipsychotic-like effect, using the CAR test. Adjunct treatment with the selective 5-HT<sub>2A</sub> receptor antagonist M100907 (0.5 mg/kg, s.c.) to a suboptimal dose of risperidone (0.25 mg/kg, i.p.) could not further enhance the antipsychotic-like effect (see Figure 12). Thus, these results indicate that increasing the 5-HT<sub>2A</sub>:D<sub>2</sub> receptor binding ratio of risperidone should not increase its antipsychotic efficacy.



**Figure 12.** Effects of risperidone (0.25 mg/kg i.p.) or M100907 (0.5 mg/kg, s.c.), alone and in combination with risperidone, on CAR behavior in rats at 20 minutes after administration of vehicle or risperidone. The results are presented as median (avoidance %)  $\pm$  semi-interquartile range. Animals are serving as their own control in a change-over design (Li, 1964).

## 4.2 Atypicality of asenapine vs. other antipsychotic drugs (Paper II)

The recently approved APD asenapine was experimentally investigated for its antipsychotic-like effect using the CAR test and the propensity for causing EPS using a catalepsy test. The effects of asenapine on regional dopamine output in the brain, i.e. in the mPFC, the NAc and the STR, were measured using *in vivo* microdialysis in awake and freely moving rats. *In vivo* voltammetry was used to assess the differential effects of asenapine on dopamine output in the core and shell subregions of the NAc. We also investigated the effect of asenapine on NMDA-induced currents in pyramidal neurons of the mPFC using intracellular electrophysiological recording *in vitro*.



**Figure 13.** Effects of asenapine on CAR behavior in rats 0 to 240 minutes after drug administration. <sup>+</sup>p<0.05 for vehicle- vs. asenapine-treated animals.

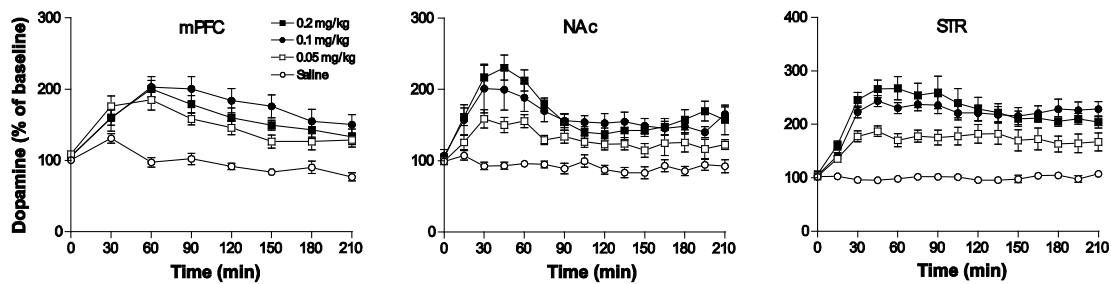
The efficacy of asenapine in the CAR test provides significant support for a potent antipsychotic action in schizophrenia (see Figure 13). Moreover, the low propensity of asenapine to cause catalepsy suggests a low EPS liability (see Table 3). Since asenapine displays a marked difference between the dose required for antipsychotic-like action in relation to that causing catalepsy, i.e. 2.5 times higher dose required, these preclinical findings contribute to define asenapine as a so-called atypical APD. Thus, the results are comparable with those obtained with e.g. risperidone (see Paper I). In contrast, for haloperidol catalepsy has been found to be near maximal, using a dose inducing a comparable suppression of CAR (Wadenberg et al., 2007). In fact, haloperidol has been demonstrated to induce marked catalepsy at a dose four times lower than that producing a sufficient antipsychotic-like effect. Clozapine on the other hand has not been shown to produce any catalepsy, even at very high doses (Wadenberg et al., 1993). Taken together, these results, which are supported by clinical studies, thus indicate that asenapine, like clozapine and risperidone but in contrast to haloperidol, has an atypical profile (Gross and Langer, 1966; Kane et al., 2010; Potkin et al., 2007).

**Table 3.** Effects of asenapine (0.1, 0.2, 0.5, 1.0 and 2.0 mg/kg, s.c.) on catalepsy in rats.

	mg/kg	Score at 30 min	Score at 60 min	Score at 120 min
Asenapine	0.1	< 2	<2	< 2
	0.2	< 2	<2	< 2
	0.5	< 2	2 ± 0.6 ***	2.5 ± 0.7 **
	1.0	2,5 ± 2,1 *	3.5 ± 1.5 ***	2 ± 1 **
	2.0	3 ± 1,2 **	2.5 ± 1.1 ***	3 ± 1.2 **

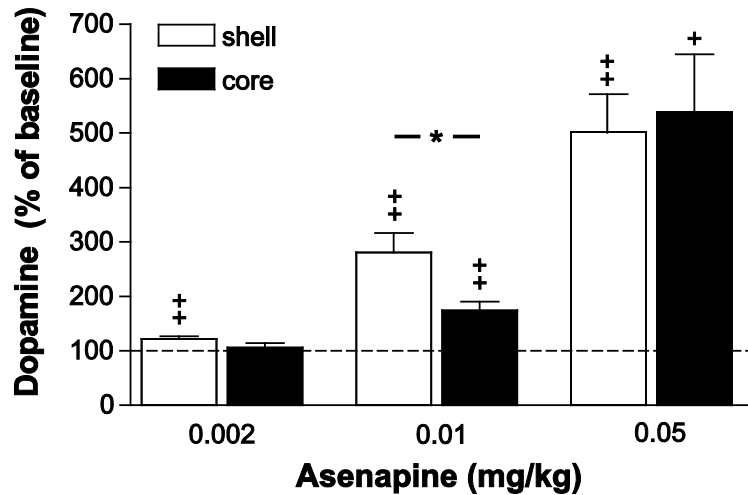
The results are shown as median ± semi-interquartile range based on observations of 8 animals per treatment group. \*  $p < 0.05$ , \*\*  $p < 0.01$ , \*\*\*  $p < 0.001$  saline vs. asenapine treated animals.

Asenapine was shown to markedly enhance the dopamine release in all areas studied, i.e. the mPFC, NAc and STR (see Figure 14). The markedly increased dopamine efflux in the mPFC produced by asenapine is in similarity with other atypical, but not typical, APDs, in particular clozapine (Kuroki et al., 1999; Li et al., 1998; Moghaddam and Bunney, 1990; Nomikos et al., 1994). Although asenapine was shown to enhance the dopamine release not only in the mPFC but also in the STR, no associated catalepsy was found, an effect profile that also can be seen with risperidone (Hertel et al., 1996). However, clozapine has a preferential effect on the mPFC vs. the STR (Hertel et al., 1996). The superiority of atypical compared to typical APDs in improving negative and cognitive functions in schizophrenia has been associated with the ability to enhance prefrontal dopamine (c.f. Introduction).



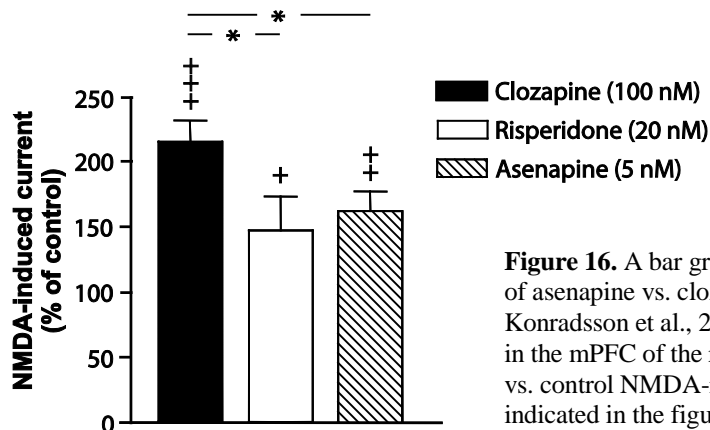
**Figure 14.** Effects of asenapine administration on dopamine output in mPFC, NAc and STR. Each point represent the mean ± S.E.M.

Moreover, the preferential effect of low doses of asenapine on dopamine output in the shell vs. the core subregion of the NAc also resembles that of atypical, in particular clozapine, but not typical APDs, such as haloperidol (see Figure 15; Marcus et al., 1996, 2000), further supporting the atypical profile of asenapine.



**Figure 15.** Over all effects of asenapine on extracellular dopamine in the shell and the core of the NAc. Data are represented as mean  $\pm$  S.E.M. The dotted line represents the baseline value (100%). <sup>+</sup>p<0.05, <sup>++</sup>p<0.01 shell or core vs. baseline. \*p<0.05 shell vs. core.

Finally, in similarity with risperidone and clozapine (20 and 100 nM, respectively; Konradsson et al., 2006), asenapine, however at a considerably lower concentration (5 nM), potentiated the NMDA-induced responses in pyramidal cells of the mPFC (see Figure 16). The maximal facilitation was similar to that of risperidone, but lower than that of clozapine. These results are in line with previous observations showing that atypical but not typical APDs facilitate NMDA-induced currents in the pyramidal cells of the mPFC (Ninan et al., 2003a). In a consecutive study, the facilitation of these glutamatergic NMDA-induced currents in pyramidal cells of the mPFC induced by asenapine was found to be mediated by prefrontal dopamine and executed via D<sub>1</sub> receptors (Jardemark et al., 2010), an effect that suggests improvement of cognitive functioning (c.f. Introduction). Asenapine, in similarity with clozapine and olanzapine but not haloperidol (Ninan et al., 2003b), was furthermore found to reverse the PCP-induced blockade of cortical NMDA-induced currents in pyramidal cells, confirming a cognitive improving effect of asenapine (Jardemark et al., 2010). This conclusion is directly supported by behavioral studies demonstrating reversal of PCP-induced cognitive impairment by asenapine, an effect shown to be mediated via D<sub>1</sub> receptors (Elsworth et al., 2012; Snigdha et al., 2011).



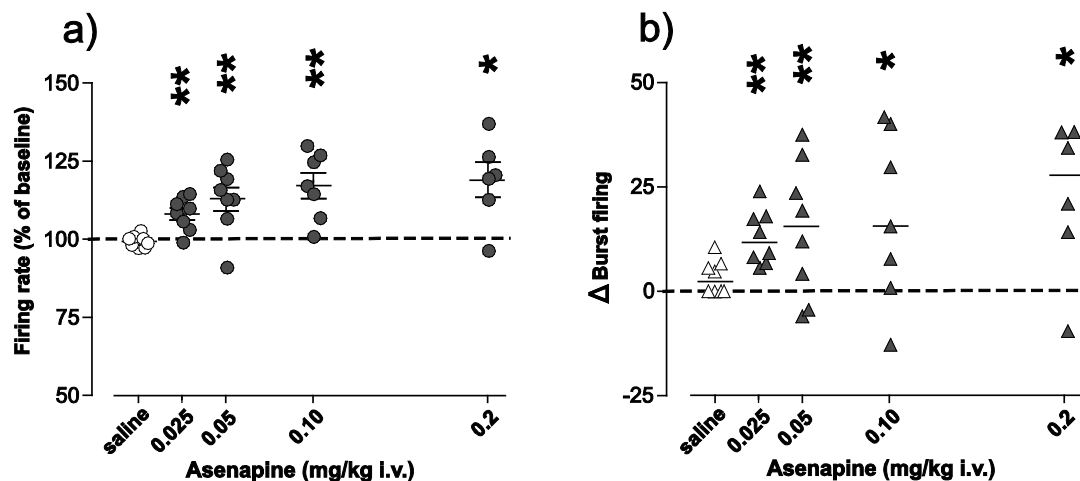
**Figure 16.** A bar graph illustrating the maximal effect of asenapine vs. clozapine and risperidone (see Konradsson et al., 2006) on NMDA-induced currents in the mPFC of the rat. <sup>+</sup>p<0.05, <sup>++</sup>p<0.01, <sup>+++</sup>p<0.001 vs. control NMDA-induced currents. \*p<0.05 as indicated in the figure.

Taken together, our results provide new insights in the mechanism of action of the novel APD asenapine, which thus was found to display an atypical profile. Our data specifically suggest that asenapine may exhibit a potent antipsychotic activity in spite of a very low EPS liability. Its ability to increase both dopaminergic and glutamatergic transmission in rat mPFC suggests that asenapine may have an advantageous effect not only on positive symptoms in patients with schizophrenia, but also on negative and cognitive symptoms.

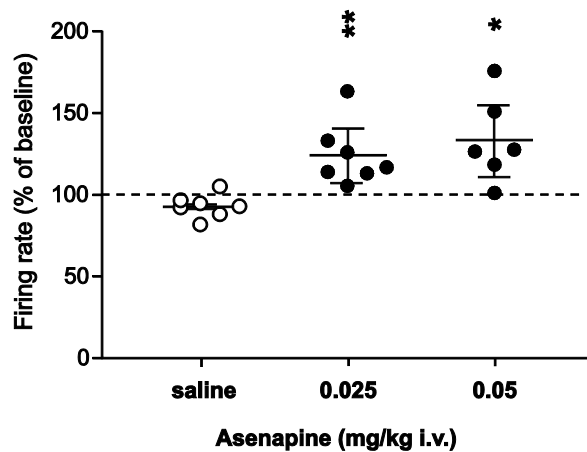
### 4.3 Effects of asenapine on cortical monoamine output in brain. Differential effects on cortical and subcortical release of dopamine (Paper III)

Using single-cell recording techniques, we here examined the effects of systemic asenapine on the firing activity of mesocorticolimbic dopaminergic neurons in the VTA and noradrenergic neurons in the LC. Furthermore, using *in vivo* microdialysis, we investigated the effects of systemic administration of asenapine on cortical noradrenaline and serotonin output. We also studied the effects of local administration of asenapine, using reverse dialysis, on cortical dopamine, noradrenaline and serotonin output and dopamine output in the NAc. Finally, we used application of TTX into the VTA, which blocks neuronal activity in dopamine neurons, to further analyze the mode of action of asenapine in the mPFC vs. the NAc.

Intravenous asenapine dose-dependently increased both dopaminergic and noradrenergic neuronal firing (see Figures 17 and 18).

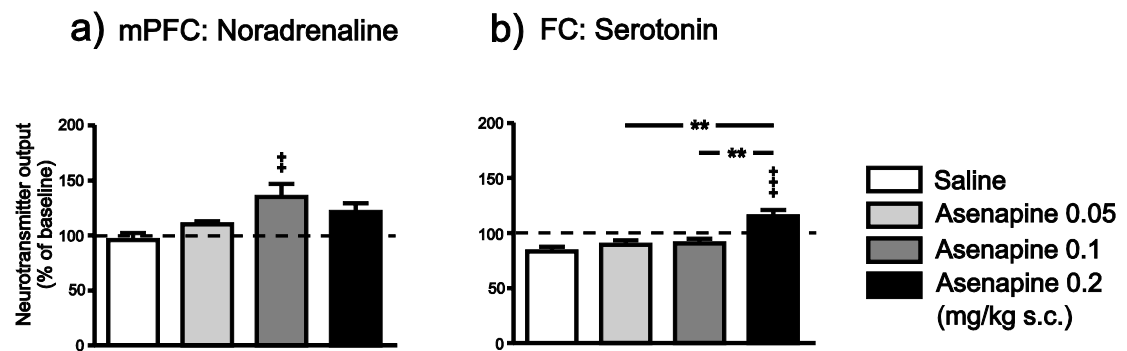


**Figure 17.** Effects of cumulative doses of asenapine 0-0.2 mg/kg, i.v. (a, b) on the firing pattern of dopamine cells in the VTA in anesthetized rats. **a)** Firing rate for each cell is represented by a circle, and mean  $\pm$  SEM are indicated by horizontal lines. **b)**  $\Delta$  Burst firing for each cell is represented by a triangle, and median is indicated by a horizontal line. \* $p < 0.05$ , \*\* $p < 0.01$  compared with saline.



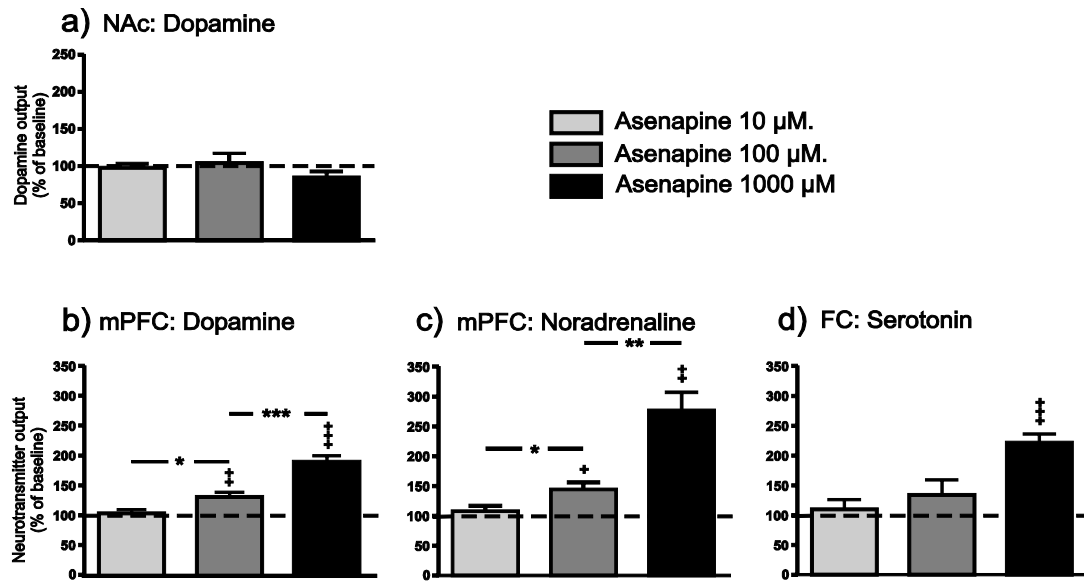
**Figure 18.** Effects of cumulative doses of asenapine (0-0.05 mg/kg, i.v.; n=6-7) on firing rate of noradrenaline cells in the LC in anesthetized rats. Firing rate for each cell is represented by a circle, and mean  $\pm$  SEM are indicated by horizontal lines. \* $p < 0.05$ , \*\* $p < 0.01$  compared with saline.

In similarity with its effects on accumbal and cortical dopamine (see above), asenapine also increased cortical noradrenaline and serotonin output following systemic administration (see Figure 19).



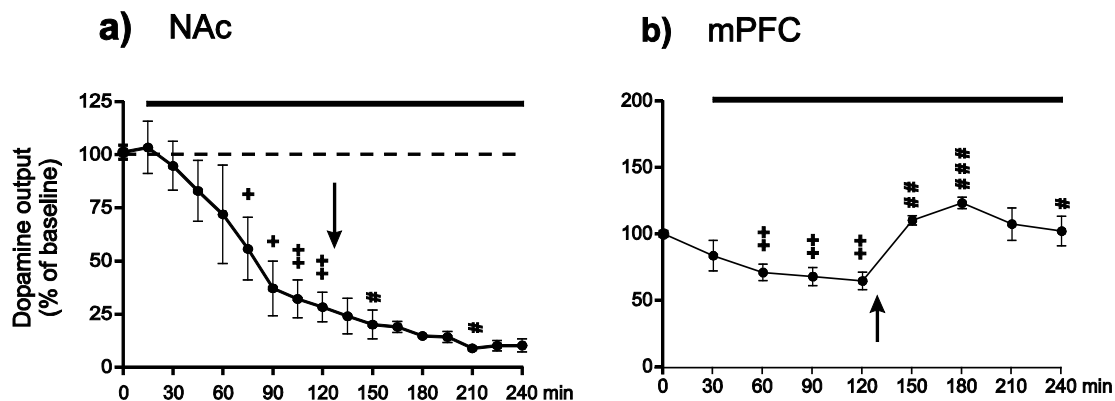
**Figure 19.** Overall effects of systemic administration of asenapine (0.05, 0.1, and 0.2 mg/kg, s.c.) on **a)** noradrenaline output in the mPFC and **b)** serotonin output in the FC in awake, freely moving rats. Bars represent mean  $\pm$  SEM percent of baseline level over 150 min following asenapine administration. The dotted line represents the baseline value (100%). ++ $p < 0.01$ , +++ $p < 0.001$  saline vs. asenapine treated animals. \*\* $p < 0.01$  between treatment groups.

Local administration of asenapine did not affect dopamine output in the NAc, but still increased cortical dopamine, noradrenaline, and serotonin output (see Figure 20).



**Figure 20.** Effects of local infusion of asenapine (10, 100, and 1000  $\mu$ M) on **a)** dopamine output in the NAc, **b)** dopamine output in the mPFC, **c)** noradrenaline output in the mPFC, and **d)** serotonin output in the FC. Each bar represents mean  $\pm$  SEM percent of baseline level over 60 min following asenapine infusion. The dotted line represents the baseline value (100%). + $p$ <0.05, ++ $p$ <0.01, +++ $p$ <0.001 each concentration vs. baseline level. \* $p$ <0.05, \*\* $p$ <0.01, \*\*\* $p$ <0.001 between different concentrations.

TTX perfusion into the VTA blocked accumbal but only partially prefrontal cortical dopamine outflow induced by systemic administration of asenapine (see Figure 21).



**Figure 21.** Effects of TTX (1.0  $\mu$ M) perfusion of the VTA and concomitant systemic administration of asenapine (0.2 mg/kg, s.c.) on extracellular **a)** dopamine concentrations in the NAc and **b)** dopamine output in the mPFC. Horizontal bar indicates TTX infusion, and arrow indicates asenapine administration, respectively. Each point represents the mean  $\pm$  SEM percent of baseline. The dotted line represents the baseline value (100%). + $p$ <0.05, ++ $p$ <0.01 compared to baseline level. # $p$ <0.05, ### $p$ <0.01, #### $p$ <0.001 compared with sample immediately preceding the injection of asenapine.



Our results propose that, whereas the increased dopamine outflow in the NAc caused by systemic asenapine is dependent on, and mediated by, the increased dopamine neuronal activity, the facilitation of prefrontal dopamine outflow by asenapine is largely independent on nerve impulse activity and instead depends on a local action at the nerve terminal level. This conclusion is supported by the finding that after intra-VTA application of TTX a markedly reduced dopamine outflow in the NAc but only partially reduced dopamine outflow in the mPFC was seen, and that TTX essentially abolished the effect of systemic asenapine on dopamine efflux in the NAc but not in the mPFC. Moreover, locally administered asenapine increased dopamine outflow in the mPFC, but had no significant effect in the NAc, which further supports this conclusion. In view of the high affinity of asenapine for 5-HT<sub>2A/2C</sub> receptors and  $\alpha_2$ -adrenoceptors (Schotte et al., 1996; Shahid et al., 2009), as well as the effects of selective ligands for these receptors on dopamine release in the PFC vs. the NAc (Hertel et al., 1999a,b; Ichikawa et al., 2001b; Liégeois et al., 2002), antagonist activity at these receptors seems likely to contribute to the increased prefrontal dopamine outflow induced by asenapine. These mechanisms may also help explain the concomitantly increased prefrontal noradrenaline outflow and, to some extent, the increase in cortical serotonin output. The finding that asenapine augments the availability of both cortical noradrenaline and serotonin, which represent effects shared by a range of antidepressant drugs, furthermore supports its utility for the treatment of depressive and/or negative symptoms in schizophrenia as well as depression as such.

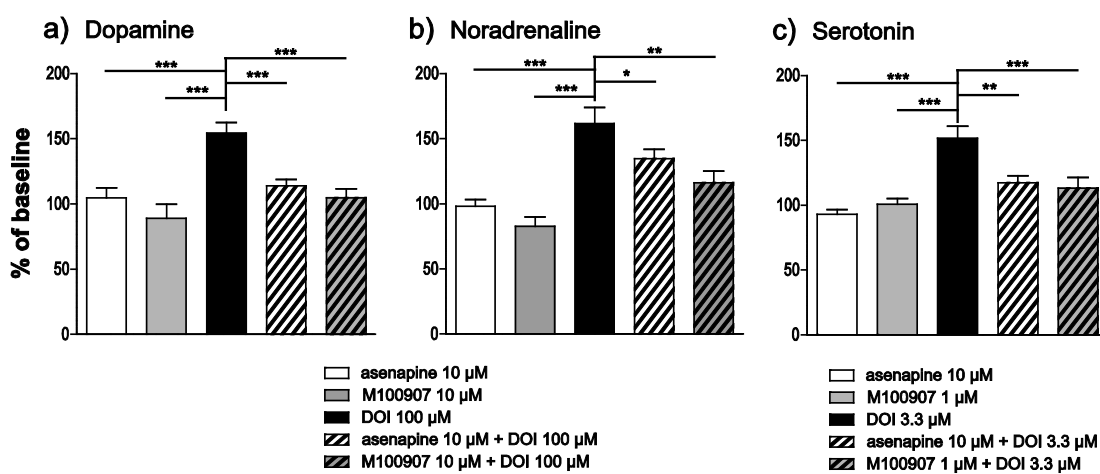
Taken together, these data and our previous work demonstrate a specific pharmacologic profile of asenapine, as well as some of its similarities with clozapine, especially with regard to its effects in the PFC, which may have bearing on its cognitive enhancing effects. As impairment of working memory often represents a major symptom not only in schizophrenia and bipolar disorder, but also in MDD, the potential utility of low dose asenapine in the treatment of MDD is further supported. Additionally, our results from the electrophysiological experiments *in vivo*, showing that very low doses of asenapine may enhance the excitability of the reward-related dopaminergic neurons in the VTA, substantiate this contention. The data which show that very low doses of asenapine also stimulates the firing of brain noradrenergic neurons in the LC, an effect shared by certain antidepressant drugs, such as mianserin (Engberg and Svensson, 1980), provide further evidence in this direction.

#### **4.4 Effects of asenapine on cortical monoamine output; role of 5-HT<sub>2A</sub> receptor- and $\alpha_2$ -adrenoceptor-mediated mechanisms (Paper IV)**

The potential role of  $\alpha_2$ -adrenoceptor and 5-HT<sub>2A</sub> receptor blockage in the effects of e.g. risperidone and clozapine has previously been discussed. Notably, asenapine exhibits high affinity for 5-HT<sub>2A</sub> receptors and  $\alpha_2$ -adrenoceptors (see section 1.11). Since our previous data indicate that the monoaminergic release by asenapine may to a

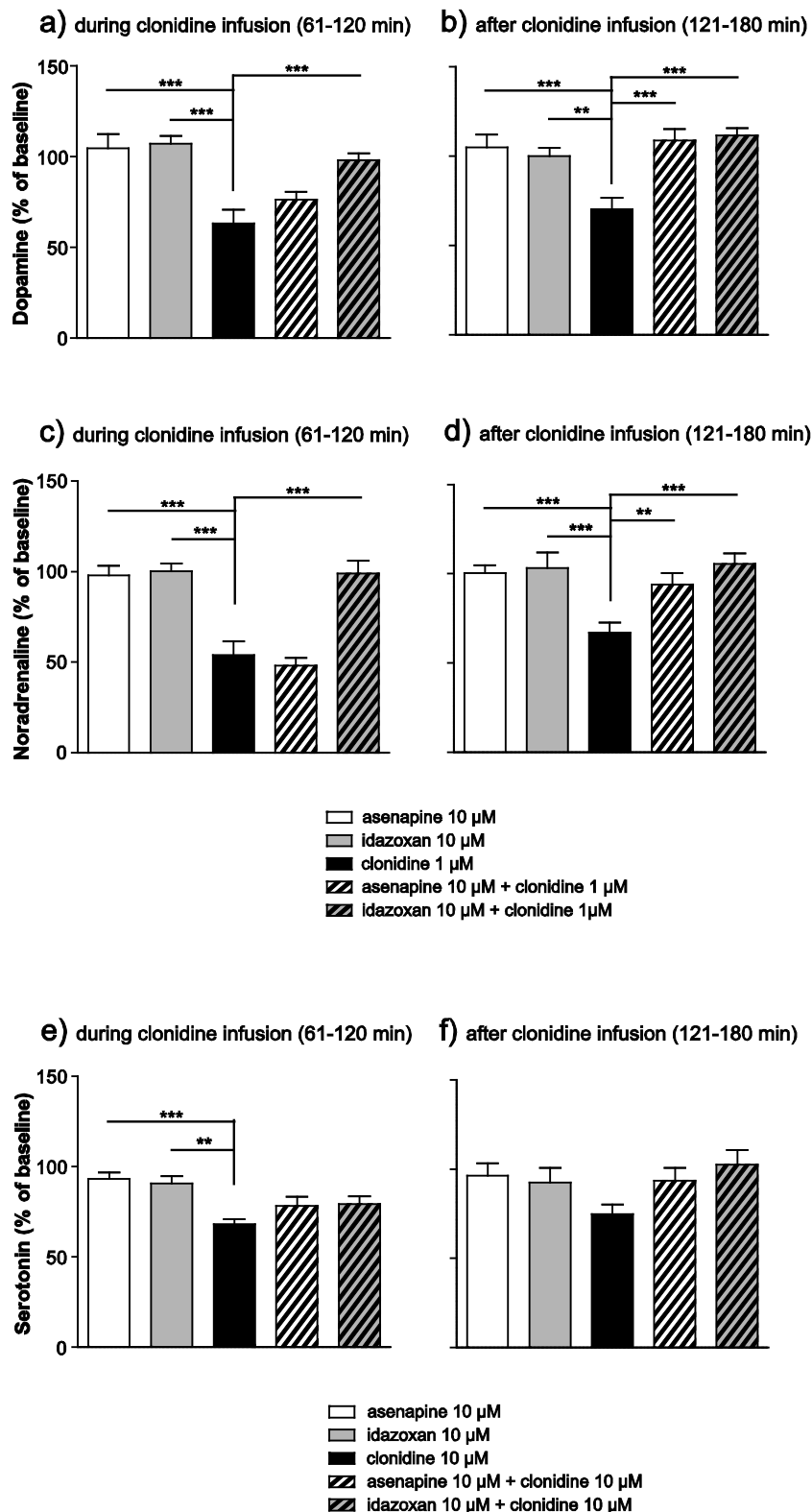
significant extent be mediated via local mechanisms in the mPFC, we here specifically investigated the role of local 5-HT<sub>2A</sub> receptors and  $\alpha_2$ -adrenoceptors for these effects. Reverse microdialysis *in vivo* with local application into the mPFC of selective receptor ligands were used.

Intracortical administration of DOI, a selective 5-HT<sub>2A</sub> receptor agonist, increased the monoamine output in the mPFC, effects that were reversed by concomitant administration of the selective 5-HT<sub>2A</sub> receptor antagonist M100907 or asenapine (see Figure 22). These results indicate a potent 5-HT<sub>2A</sub> receptor affinity by asenapine *in vivo* as well as an involvement of 5-HT<sub>2A</sub> receptors in the regulation of the monoamine release of asenapine.



**Figure 22.** The 5-HT<sub>2A</sub> antagonistic effect of asenapine on extracellular concentration of a) dopamine, b) noradrenaline and c) serotonin output in the mPFC. The mean transmitter release during DOI infusion, that is, 61–120 min. Data are presented as % of baseline (mean  $\pm$  SEM). \*p < 0.05, \*\*p < 0.01, and \*\*\*p < 0.001 comparisons vs. DOI infusion alone.

Intracortical administration of the selective  $\alpha_2$ -adrenoceptor agonist clonidine significantly decreased the monoamine release in the mPFC (see Figure 23). Application of the  $\alpha_2$ -adrenoceptor antagonist idazoxan blocked the clonidine-induced reduction of dopamine and noradrenaline release. However, the effect on serotonin was less pronounced. Simultaneous infusion of asenapine did not acutely block the clonidine-induced decrease in transmitter release. However, following termination of the clonidine infusion the transmitter release remained at a low level. During this interval, i.e. the following hour after the clonidine infusion, intracortical infusion of asenapine restored the catecholamine release to baseline level, indicating an  $\alpha_2$ -adrenoceptor blocking effect of asenapine which was somewhat delayed. When given alone, the infusion of asenapine, M100907 or idazoxan, in the concentrations used, did not affect monoamine release.



**Figure 23.** The  $\alpha_2$ -antagonistic effect of asenapine on extracellular concentration of **a–b**) dopamine, **c–d**) noradrenaline, and **e–f**) serotonin output in the mPFC. **a, c, e**) The mean transmitter release during clonidine infusion, that is, 61–120 min. **b, d, f**) The mean transmitter release after clonidine infusion was completed, that is, 121–180 min. Data are presented as % of baseline (mean  $\pm$  SEM). \* $p < 0.05$ , \*\* $p < 0.01$ , and \*\*\* $p < 0.001$  comparisons vs. clonidine infusion alone.

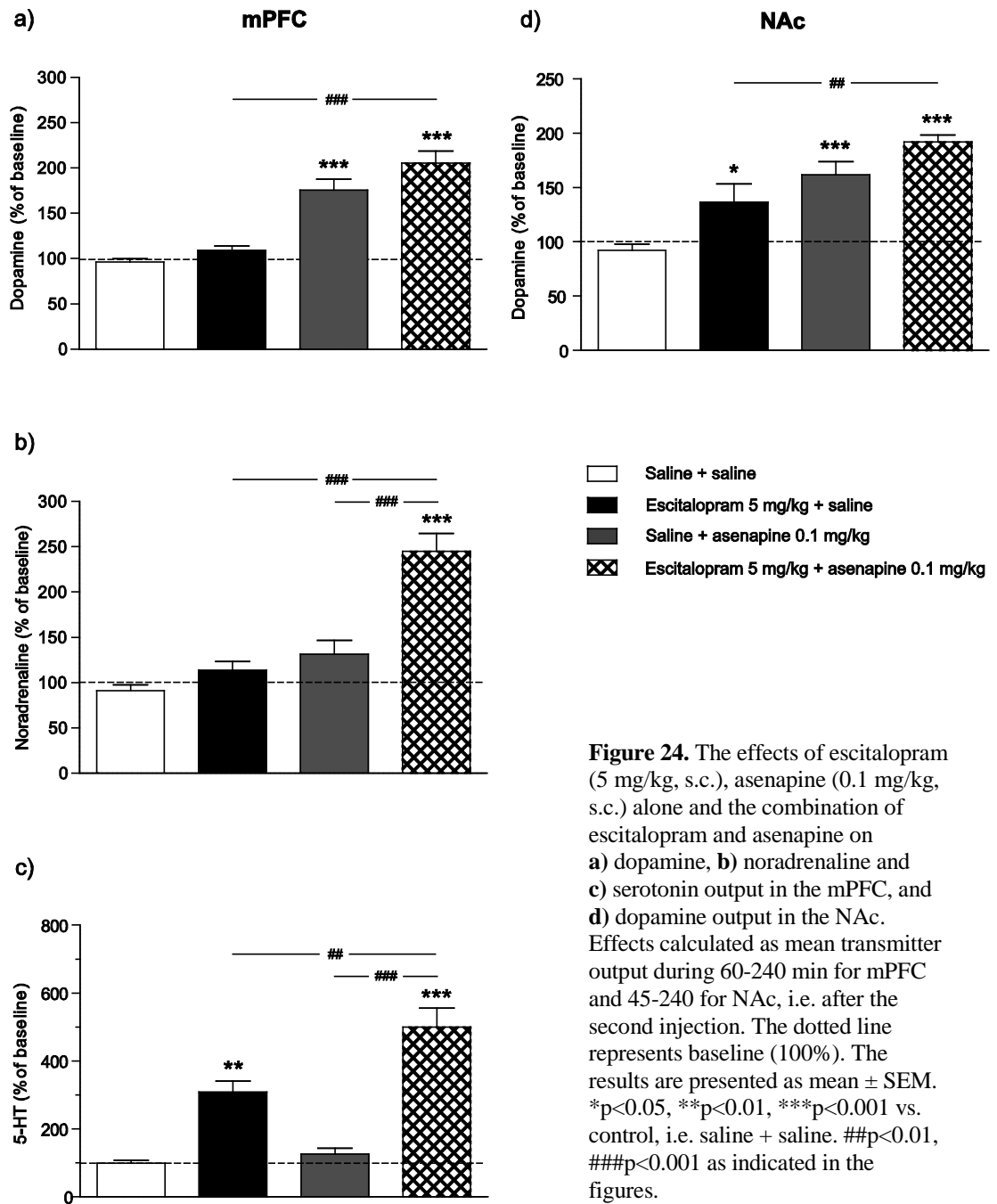
In summary, these findings demonstrate that asenapine possesses an antagonistic affinity for local 5-HT<sub>2A</sub> and  $\alpha_2$ -adrenergic receptors at the nerve terminals in the mPFC. Thus, 5-HT<sub>2A</sub> receptor antagonism and  $\alpha_2$ -adrenoceptor blockage induced by asenapine in the mPFC may contribute to enhance prefrontal monoamine release *in vivo* and, secondarily, to its effect on positive and negative symptoms as well as pro-cognitive and antidepressant profile.

#### **4.5 Adjunctive treatment with asenapine augments the escitalopram-induced effects on monoaminergic and glutamatergic NMDA as well as AMPA receptor-mediated transmission in the medial prefrontal cortex. (Paper V)**

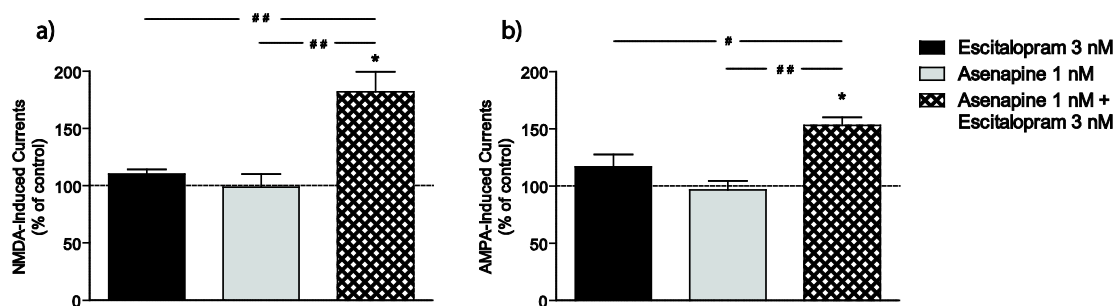
In recent years, the beneficial effects of low to moderate doses of atypical APDs as add-on to SSRIs in the management of treatment-resistant depressive symptoms in MDD as well as bipolar disorder, which may generate an enhanced antidepressant effect with a faster onset of action, have been increasingly supported (see section 1.3.4 and 1.4.4). According to previous studies, the mechanism for this augmentation strategy may involve activation of cortical catecholamine output (Dremencov et al., 2007; Koch et al., 2004; Marcus et al., 2012; Zhang et al., 2000). In addition, an associated facilitation of glutamatergic NMDA receptor-mediated transmission in pyramidal cells of the mPFC, has been observed (Marcus et al., 2012). Recently, the very rapid and potent antidepressant action of the NMDA receptor antagonist ketamine as well as scopolamine has been shown to be dependent on activation of prefrontal AMPA receptors (Duman, 2011; Li et al., 2010).

We here investigated whether the atypical APD asenapine may further enhance the effect of the SSRI escitalopram on monoamine availability in the mPFC as well as dopamine availability in the NAc, using *in vivo* microdialysis in freely moving rats. To study the glutamatergic NMDA- and AMPA-induced currents of pyramidal neurons of the mPFC, intracellular electrophysiological recording *in vitro* was used.

Low doses of asenapine (0.05 and 0.1 mg/kg) as add-on to escitalopram were found to significantly increase the availability of monoamines in the mPFC (see Figure 24 a-c). In the NAc, escitalopram increased the extracellular levels of dopamine, which were further enhanced by adjunctive asenapine (see Figure 24 d).



Moreover, the combination of asenapine and escitalopram in low concentrations, which had no effects when given alone, was found to facilitate both NMDA- and AMPA-induced currents in pyramidal cells of the mPFC (see Figure 25). Recent data indicate that also the combination of olanzapine and fluoxetine, in concentrations that were without effect when given alone, facilitates AMPA-induced currents (Björkholm et al., 2012). As this drug combination is known to improve depressive symptoms with a fast onset in both bipolar disorder and treatment-resistant MDD (Dubé et al., 2007; Nelson and Papakostas, 2009; Shelton et al., 2005), our data suggest that a similar clinical outcome with a combination of asenapine and an SSRI would be expected.



**Figure 25.** Effects on a) NMDA- and b) AMPA-induced currents in pyramidal cells of the rat mPFC of a combination of asenapine and escitalopram at 30 min after drug application. The results are presented as mean  $\pm$  SEM. \* $p < 0.05$  vs. control NMDA-induced currents. ## $p < 0.01$  as indicated in the figure. The holding potential was -60 mV.

Our results generally support the notion that the effects of adjunctive treatment of atypical APDs to SSRIs in treatment-resistant MDD may be related to an increase of prefrontal catecholaminergic output. The enhanced prefrontal monoamine release and the concomitant facilitation of NMDA-induced currents in the mPFC provide a rationale for potential pro-cognitive as well as enhanced antidepressant effects of the combination of asenapine and escitalopram. Finally, our novel observation demonstrates that this drug combination, as previously shown with ketamine and scopolamine, also markedly facilitates AMPA receptor-mediated responses in the mPFC of the rat. This effect may thus contribute to explain the potent and rapidly enhanced antidepressant effect that has been seen clinically following add-on treatment of atypical APDs with SSRIs in treatment-resistant depression (see section 1.3.4 and 1.4.4). Needless to say, further studies are needed to validate this notion.

## 5 SUMMARY AND CONCLUDING REMARKS

In the present set of experimental studies we demonstrate that additional blockage of  $\alpha_2$ -adrenoceptors, but not 5-HT<sub>2A</sub> receptors, may further enhance the antipsychotic-like effect of low doses of risperidone without increasing EPS liability. These behavioral changes were accompanied by an increase in prefrontal dopaminergic and glutamatergic NMDA receptor-mediated transmission.

Subsequently, asenapine was shown to possess a potent antipsychotic-like effect without any associated high risk of EPS and like other atypical, but not typical APDs asenapine was shown to markedly increase cortical dopamine release as well as facilitate glutamatergic NMDA receptor-mediated transmission in pyramidal cells in the mPFC. In similarity with other atypical APDs, asenapine also preferentially increased dopamine release in the shell relative to the core subregion of the NAc. Furthermore, whereas the dopamine release induced by asenapine in the NAc was dependent on the concomitant stimulation of neuronal firing in the dopamine neurons originating in the VTA, the cortical dopamine release was largely regulated via local mechanisms in the nerve terminal region. When further investigating the effects of asenapine, we found that this drug also augments the release of noradrenaline and serotonin. Moreover, asenapine was shown to exert a potent antagonistic activity at  $\alpha_2$ -adrenergic and 5-HT<sub>2A</sub> receptors *in vivo*, an effect found to be involved in its regulation of cortical monoamine release.

Finally, our data demonstrate that add-on treatment with asenapine in combination with the SSRI escitalopram enhances the monoamine output in the mPFC. Moreover, the combination of asenapine and escitalopram, in concentrations that had no effects when given alone, was found to facilitate both glutamatergic NMDA- as well as AMPA-mediated responses in the pyramidal cells of the mPFC, supporting a clinical utility of asenapine as adjunctive treatment in depressive disorders.

Taken together, these studies contribute to establish asenapine as an atypical APD and help explain, at the neurobiological systems level in the brain, some of the clinical characteristics and utilities of the drug. The data also support a cognitive enhancing effect of asenapine, as well as an antidepressant potential, which in view of the structural similarities between asenapine and mianserin and mirtazapine is not surprising *per se*. Although asenapine like most atypical APDs is a potent 5-HT<sub>2</sub> receptor antagonist, this part of its pharmacological profile can obviously not serve to differentiate asenapine from other atypical APDs. Rather, its high affinity to a number of serotonergic receptors may be of importance in this regard. Given a substantial set of studies, both clinical and preclinical, the  $\alpha_2$ -adrenoceptor antagonistic effect of asenapine may underlie some of its apparently rather broad clinical utility. A recent meta-analysis (Hecht and Landy, 2012) provides substantial support for this view, in particular as regards its effects on negative symptoms and cognitive impairment in schizophrenia. Since a previous clinical study in patients with MDD (Sanacora et al., 2004) reported that addition of an  $\alpha_2$ -adrenoceptor antagonist to fluoxetine seemed to both increase the number of responders and hasten the antidepressant response, the  $\alpha_2$  receptor antagonistic action of asenapine may not only contribute to augment the antidepressant action of SSRIs, but also reduce the time to onset of the effect. This notion has recently been supported by studies of early changes in neuroplasticity-

related genes following chronic antidepressant treatment (Serres et al., 2012), where a combined  $\alpha_2$ -adrenoceptor antagonist/SNRI generated a more rapid response than a potent SNRI without  $\alpha_2$ -adrenoceptor blocking action i.e. venlafaxine.

Our novel observation that a combination of asenapine and an SSRI, escitalopram, just like the rapidly acting antidepressants ketamine and scopolamine, may facilitate AMPA receptor-induced responses in the mPFC, provides further support for an antidepressant potential of asenapine as adjunctive treatment in MDD. Moreover, previous experimental data have shown a dose-dependent antidepressant effect of an AMPA-receptor potentiation that during subchronic treatment for three weeks was comparable to that of fluoxetine, but showed a clearly faster onset of action, i.e. appearing within one week vs. two weeks, respectively (Knapp et al., 2002). Thus, both  $\alpha_2$ -receptor antagonism and AMPA receptor potentiation support the utility of asenapine as adjunct treatment to SSRIs in MDD as well as in bipolar depression.

Although presently a multitude of APDs are available, more treatment options are obviously needed, both for reasons of efficacy and side effect profile. In view of the partial overlap in symptoms and to some extent even underlying neurobiology between schizophrenia, bipolar disorder and MDD, drugs with a broad clinical utility may be of particular interest. This notion is further supported by the recently reported marked reduction of suicide risk following combined antipsychotic and antidepressant medication in schizophrenia (Tiihonen et al., 2012). Finally, by inference our data also suggest that increasing the  $\alpha_2:5\text{-HT}_{2A}$  receptor binding ratio of risperidone might serve to increase its utility as adjunct treatment in MDD and, like e.g. clozapine, reduce suicide risk in the treatment of schizophrenia.



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