

From DEPARTMENT OF PHYSIOLOGY AND PHARMACOLOGY

Karolinska Institutet, Stockholm, Sweden

**NOVEL PHARMACOLOGICAL  
TREATMENT ALTERNATIVES FOR  
SCHIZOPHRENIA**

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**Till Farfar**

*"Jag är ett geni"*

*Ted 3½ år*

# ABSTRACT

Antipsychotic drugs are commonly divided into typical (first generation) and atypical (second generation) antipsychotic drugs. Clinically effective doses of typical antipsychotic drugs generate a striatal dopamine D<sub>2</sub> receptor occupancy of about 70 – 80%, approaching a level that is associated with a high risk of extrapyramidal side effects. Typical antipsychotic drugs are mostly effective against positive symptoms, but have a more limited effect on and may even exacerbate negative and cognitive symptoms. In contrast, some, but not all, atypical antipsychotic drugs are clinically effective at lower D<sub>2</sub> receptor occupancy. Clozapine, which may be considered as the prototypical atypical antipsychotic drug, thus displays only around 45% D<sub>2</sub> receptor occupancy and generates virtually no extrapyramidal side effects. Compared with typical antipsychotic drugs, atypical antipsychotics have been found to improve not only positive symptoms, but also negative symptoms and cognitive impairment. Several atypical antipsychotic drugs are, however, associated with other major side effects, such as for example weight gain, diabetes, heavy sedation and even agranulocytosis. Clozapine has a broad spectrum of receptor affinities and acts as an antagonist at several dopamine receptors (i.e. D<sub>2</sub>, D<sub>3</sub> and D<sub>4</sub> receptors), but also at e.g.  $\alpha_1$  and  $\alpha_2$  adrenoceptors, 5-HT<sub>2A</sub> and 5-HT<sub>2C</sub> receptors, muscarinic and histaminergic (H<sub>1</sub>) receptors. In contrast to a typical antipsychotic drug such as haloperidol most atypical antipsychotic drugs have been found to increase dopamine in the medial prefrontal cortex of rodents. Moreover, most atypicals that generate improved cognitive function also enhance prefrontal glutamatergic transmission and increase extracellular levels of acetylcholine in the cerebral cortex. Since treatment outcome is critically dependent on the degree of cognitive impairment, the putative cognitive enhancing effects of antipsychotic drugs are of considerable interest. Generally, the currently available drugs for schizophrenia are insufficient in this regard. Therefore, the development of more effective and better tolerated antipsychotic drugs with less side effects is highly warranted. This development may be facilitated by evaluation of so-called augmentation strategies using combinations of existing antipsychotics and other psychoactive drugs, such as for example antidepressants and various selective ligands for specific neurotransmitter-related targets in the brain. In this thesis we have experimentally evaluated several augmentation strategies employing a combination of behavioral, biochemical and electrophysiological techniques.

Our results show that the addition of two antidepressant drugs, reboxetine or mianserin, significantly augment the antipsychotic-like effect induced by raclopride without affecting the raclopride-induced catalepsy, concomitant with a preferentially increased prefrontal dopamine output. We have also shown that adjunctive treatment with the selective  $\alpha_2$  adrenoceptor antagonist idazoxan when added to a typical (haloperidol) or an atypical (olanzapine) antipsychotic drug, significantly enhances the suppression of conditioned avoidance response (CAR) without increasing catalepsy and produces a substantial increase in dopamine output in the medial prefrontal cortex. Moreover, we have found that galantamine, a cognitive enhancing drug used in Alzheimer's disease, facilitates the excitability of VTA dopamine neurons via allosteric potentiation of nicotinic acetylcholine receptors of the  $\alpha_7$  subtype, and that galantamine through this mechanism, rather than through acetylcholine esterase inhibition, increases prefrontal dopamine outflow. Subsequently, we observed that addition of galantamine to raclopride can significantly enhance the raclopride-induced suppression of CAR without increasing catalepsy, effects that could not be reproduced by adjunctive treatment with donepezil, a selective acetylcholine esterase inhibitor. Our data support the view that increasing prefrontal dopamine and noradrenaline output either by blocking the noradrenaline transporter or the noradrenergic autoreceptor may represent viable strategies to augment the efficacy of antipsychotic drugs without increasing extrapyramidal side effect liability. Our previous results also provide evidence that this strategy may secondarily facilitate cortical glutamatergic transmission and cognitive functioning, e.g. working memory. Our data finally provide support for the adjunctive use of galantamine together with antipsychotic drugs in schizophrenia, not only to improve cognition and negative symptoms, but also to potentially enhance the antipsychotic effect *per se*. By inference, these results support the development of selective allosteric potentiators of  $\alpha_7$  nicotinic acetylcholine receptors for the treatment of schizophrenia.

# LIST OF PUBLICATIONS

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- III. Wadenberg ML, Wiker C, Svensson TH (2007) Enhanced efficacy of both typical and atypical antipsychotic drugs by adjunctive  $\alpha_2$  adrenoceptor blockade: experimental evidence. *International Journal of Neuropsychopharmacology*, 10(2):191-202.
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# LIST OF ABBREVIATIONS

3-MT	3-Metoxytyramine
5-HIAA	5-Hydroxyindole acetic acid
5-HT	5-Hydroxytryptamine, serotonin
ACh	Acetylcholine
AChE	Acetylcholine esterase
AChR	Acetylcholine receptor
ANOVA	Analysis of variance
cAMP	3',5'-cyclic adenosine monophosphate
COMT	Catechol-O-methyltransferase
DAG	Diacyl-glycerol
CAR	Conditioned avoidance response
DOPAC	Dihydroxyphenylacetic acid
EPS	Extrapyramidal side effects
GABA	$\gamma$ -aminobutyric acid
HPLC	High performance liquid chromatography
HVA	Homovanillic acid
ISHs	Interspike time interval histograms
i.p.	Intraperitoneally
IP <sub>3</sub>	Inositol triphosphate
LC	Locus coeruleus
L-DOPA	L-dihydroxyphenyl-alanine
mAChR	Muscarinic acetylcholine receptor
MAO	Monoamine oxidase
MHPG	3-methoxy-4-hydroxy-phenethylenglycol
NAC	Nucleus accumbens
nAChR	Nicotinic acetylcholine receptor
NMDA	<i>N</i> -methyl-D-aspartate
PCP	Phencyclidine
PFC	Prefrontal cortex
s.c.	Subcutaneously
VTA	Ventral tegmental area

# 1 INTRODUCTION

## 1.1 SCHIZOPHRENIA

Schizophrenia is one of the most debilitating psychiatric disorders and affects about 1% of the world's population. The clinical signs and symptoms of schizophrenia are very complex and display different patterns which vary widely from patient to patient. The symptoms are commonly divided into three broad clusters: positive symptoms, negative symptoms and cognitive impairment. The positive symptoms represent an addition to normal behavior, involving hallucinations (perceptual experiences not shared by others), delusions (e.g. that others can interfere with your thoughts), thought disorder, bizarre behavior and disorganized speech. The negative symptoms comprise elements that is absent from normal behavior, including anhedonia (loss of the ability of experiencing pleasure), withdrawal from social contacts, amotivation, apathy and alogia (reduced quantity or content of speech). Patients with schizophrenia also suffer from cognitive impairment such as diminished attention, memory and executive functions (i.e. the ability to plan, initiate and regulate goal-directed behaviors). The manifestation of positive symptoms is not constant in intensity or continuity, but tends to fluctuate over time, whereas negative symptoms and cognitive impairment are usually more pervasive and fluctuate less over time than positive symptoms (Fenton and McGlashan, 1991).

The onset of schizophrenia usually occurs in the late adolescence or early adulthood, somewhat earlier in men than in women. Family studies have demonstrated that susceptibility to schizophrenia is clearly related to genetic factors: among first-degree relatives the incidence of the disease is about 6-17%; in dizygotic twins about 17%; and in monozygotic twins as high as 50% (Lewis and Lieberman, 2000). Linkage studies have shown that a polymorphism (Val<sup>108/158</sup>Met) in the catechol-O-methyltransferase (COMT) gene, which increases the prefrontal dopamine catabolism, is associated with a slightly increased risk of developing schizophrenia (Egan et al., 2001). In addition, impaired P50 auditory sensory gating, which has been postulated to contribute to cognitive impairment and even hallucinations in schizophrenia (Adler et al., 1998) has been linked to the  $\alpha 7$  nicotinic acetylcholine (ACh) receptor (nAChR) gene (Freedman et al., 1997). Schizophrenia has generally been claimed to be independent of geographic, cultural and socio-economic variables (Carpenter and Buchanan, 1994; Sartorius et al., 1986). However an increased incidence of schizophrenia has been found in some ethnic minority populations such as second generation Afro-Caribbean people in the UK (Boydell et al., 2001) and Dutch Antillean and Surinamese immigrants in Holland (Selten et al., 1997). In addition, schizophrenia is slightly more common in people born in cities and affects more often men than women (see McGrath, 2006). Furthermore, the risk of developing schizophrenia is also increased by pre- and peri-natal events, including premature birth, low birth weight and obstetric complications (Cannon et al., 2002), as well as if the mother experiences influenza during pregnancy (Takei et al., 1996). Approximately one in 10 patients with schizophrenia will commit suicide each year and about 50% of the patients will make suicide attempts during their lifetime (Meltzer, 2002). While the pathophysiology of schizophrenia remains unclear, several

neurotransmitter systems have been suggested to be implicated, e.g. dopamine, glutamate and ACh. Among these, the dopamine system has received most attention.

## 1.2 THE DOPAMINERGIC SYSTEM

### 1.2.1 DOPAMINE

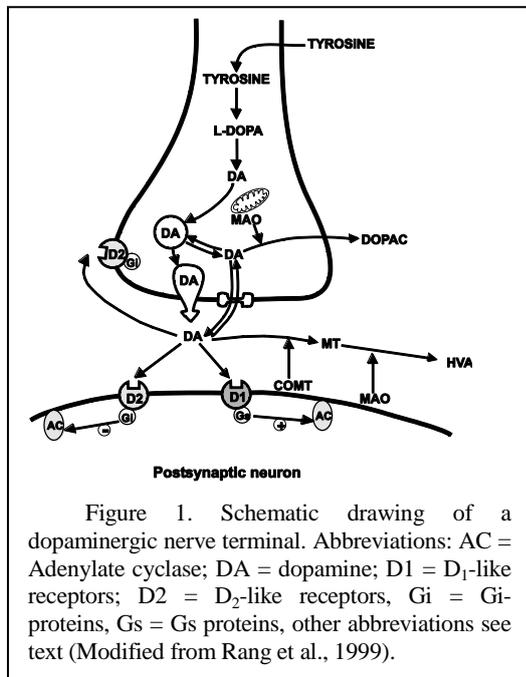


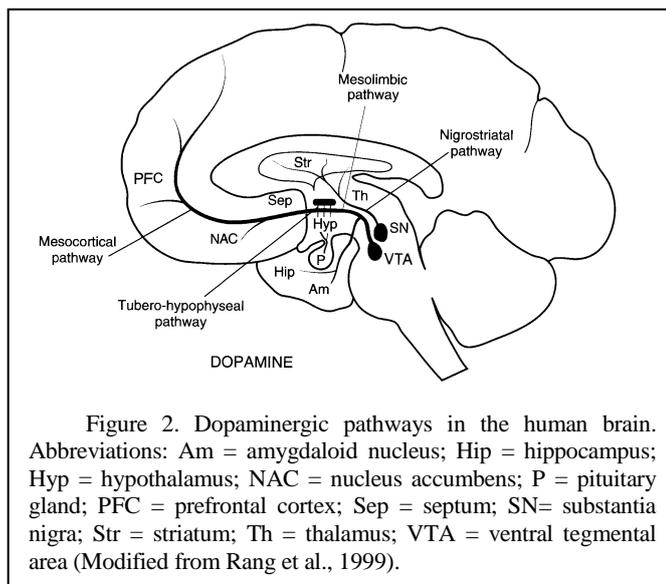
Figure 1. Schematic drawing of a dopaminergic nerve terminal. Abbreviations: AC = Adenylate cyclase; DA = dopamine; D1 = D<sub>1</sub>-like receptors; D2 = D<sub>2</sub>-like receptors; Gi = Gi-proteins, Gs = Gs proteins, other abbreviations see text (Modified from Rang et al., 1999).

Catecholamines are neurotransmitters (dopamine, noradrenaline and adrenaline), named after their chemical structure, i.e. they all contain a catechol group (a benzene ring possessing two adjacent hydroxyl group) and a side chain of ethylamine or one of its derivatives. The amino acid tyrosine, derived from food proteins, is the original precursor of the catecholamines. Tyrosine is taken up from the bloodstream by an active transport mechanism across the blood brain barrier into the catecholaminergic neuron. First, tyrosine is converted to L-dihydroxyphenyl-alanine (L-DOPA) by the enzyme tyrosine hydroxylase, which is the rate limiting enzyme in the catecholamine synthesis. L-DOPA is

decarboxylated by the enzyme L-aromatic amino acid decarboxylase to dopamine, the final product in dopaminergic neurons. Dopamine is stored in vesicles in the nerve terminal. The release of dopamine from the nerve terminals is a calcium-dependent process initiated by nerve impulse activity. Dopamine is also co-released with noradrenaline from noradrenergic neurons (Devoto et al., 2001; Devoto et al., 2003). Released dopamine is transported back into the neuron via a dopamine-selective transporter, but can also be transported similarly by the noradrenaline transporter into noradrenergic neurons (Carboni et al., 1990). Reuptake is the main mechanism for inactivation of the released transmitter. Extravesicular dopamine is intracellularly metabolized by the enzyme monoamine oxidase (MAO) to dihydroxyphenylacetic acid (DOPAC). Two different forms of MAO exists in human and rat brain, MAO-A and MAO-B. MAO-A has a substrate preference for noradrenaline and serotonin, whereas dopamine appears to be an equally good substrate for both MAO-A and MAO-B. Extracellular dopamine is degraded by COMT to 3-methoxytyramine (3-MT), which is further degraded to homovanillic acid (HVA).

### 1.2.2 THE DOPAMINERGIC SYSTEMS IN THE BRAIN

The dopamine neurons in the brain are organized into four systems: the nigrostriatal, the mesolimbic, the mesocortical and the tuberoinfundibular dopaminergic systems. The *nigrostriatal* dopaminergic system originates in the substantia nigra and projects primarily to the striatum i.e. the caudate and the putamen (Andén et al., 1964). This system is involved in motor control, and degeneration of dopaminergic neurons originating in the substantia nigra



causes Parkinson's disease (Carlsson, 1959; see Hornykiewicz, 1970). In addition, inhibition of nigrostriatal dopaminergic neurotransmission system by antipsychotic drugs is associated with extrapyramidal side effects (EPS) including parkinsonism (see Farde et al., 1992). The efferent projections from the ventral tegmental area (VTA) have a more diverse distribution. Based on the different projections, this system is further subdivided into the *mesolimbic* and *mesocortical* dopamine systems. The mesolimbic pathway projects to several limbic areas of the brain, including the nucleus accumbens (NAC), the amygdala and the olfactory tubercle (see Moore and Bloom, 1978). This system plays a role in emotional expression, motivation and reward (see Le Moal and Simon, 1991). The mesocortical dopamine system most densely projects to the prefrontal cortex (PFC) (see Moore and Bloom, 1978). The PFC is generally involved in working memory, motivation, organization, attention and social behaviors, and dysfunction of the prefrontal dopaminergic system may be importantly involved in the negative symptoms and cognitive deficits in schizophrenia (see Goldman-Rakic and Selemon, 1997). Finally, the *tuberoinfundibular* dopaminergic system originates in the hypothalamus and projects to the pituitary stalk (see Moore and Bloom, 1978). This system is involved in endocrine control and its inhibition by antipsychotic drugs causes an increased prolactin secretion, resulting in side-effects such as sexual dysfunction, infertility, gynecomastia and galactorrhea (Haddad and Wieck, 2004).

### 1.2.3 ELECTROPHYSIOLOGICAL PROPERTIES OF DOPAMINERGIC NEURONS

The firing characteristics of dopamine neurons have been well characterized by electrophysiological techniques *in vivo* and *in vitro*. Spontaneously firing dopamine neurons have long action potentials (2-5 ms) and display two different characteristic firing modes *in vivo*; single spike firing and burst firing (Bunney et al., 1973b; Grace and Bunney, 1984a; Grace and Bunney, 1984b; Wang, 1981). Single spike firing is a relatively regular, low frequency firing pattern i.e. between 1-10 Hz. In contrast, burst firing is typically recognized as a transient high frequency discharge of multiple action potentials followed by an inactive period before spiking recommences. Dopamine neurons studied *in vitro* also display a spontaneous but relatively slow and highly regular firing activity of 0.5-4.0 Hz and unlike the *in vivo* situation, midbrain dopamine neurons fail to exhibit a burst firing pattern *in vitro* (Johnson and North, 1992; Kita et al., 1986; Lacey et al., 1987; Llinas et al., 1984), thus clearly demonstrating the important role of afferent input for the cell firing pattern. Dopamine neurons in the VTA and the substantia nigra do not differ markedly in their basal physiological properties or in their

response to pharmacological agents *in vitro*, but display different physiological activity as well as responsivity to certain pharmacological challenges *in vivo* (Grenhoff et al., 1990; Grenhoff et al., 1988b), again underlining the importance of afferent regulation for the function of dopaminergic neurons.

#### 1.2.4 REGULATION OF DOPAMINERGIC NEUROTRANSMISSION

Dopaminergic neurotransmission is regulated by auto-inhibitory mechanisms as well as by various afferent inputs (see Kalivas, 1993; Moore et al., 1999). The feedback regulation of the electrophysiological activity of dopamine neurons in the VTA and substantia nigra is largely executed by D<sub>2</sub>-like receptors located in the somatodendritic region of the dopamine neurons (Bunney et al., 1973a; Bunney et al., 1973b). Thus, dopamine released from dendrites or axon collaterals activates the D<sub>2</sub> autoreceptors, thereby increasing a membrane potassium conductance which hyperpolarizes the cell, resulting in a decrease in the basal firing rate (Lacey et al., 1987). D<sub>2</sub>-like autoreceptors located on dopaminergic nerve-terminals may also regulate both synthesis and release of dopamine (see Cooper et al., 2003; Imperato and Di Chiara, 1988; Kehr et al., 1972). The mesocortical dopamine neurons have been shown to lack or have a reduced number of such impulse-modulating autoreceptors (Chiodo et al., 1984) and also to show a differential response to several classes of drugs (Arborelius et al., 1993; see e.g. Murase et al., 1993), pointing at several physiological and pharmacological differences between subcortically and cortically projecting dopamine neurons.

A glutamatergic innervation of the VTA, originating in the prefrontal cortex (Christie et al., 1985; Sesack and Pickel, 1992), serves as an excitatory input to the dopamine neurons (Gariano and Groves, 1988; Grenhoff et al., 1988a; Svensson and Tung, 1989). The glutamatergic input regulates the firing characteristics of the dopamine neurons, and in response to activation of glutamatergic *N*-methyl-D-aspartate (NMDA) receptors on their cell bodies, a marked increase in burst firing is observed (Charley et al., 1991; Chergui et al., 1993; Murase et al., 1993). The inhibitory  $\gamma$ -aminobutyric acid (GABA) input to dopamine cells in the VTA is mediated both via afferents from other brain regions and GABAergic interneurons within the nucleus (see Kalivas, 1993), which, in turn, also receives a glutamatergic input from the prefrontal cortex (Carr and Sesack, 2000).

Experimental data indicate that central noradrenaline activity modulates mesolimbocortical dopamine activity via different mechanisms. Thus, early biochemical studies indicated that the activity of dopamine neurons in the VTA decreased following selective destruction of noradrenergic fibers innervating the nucleus, whereas drugs that increase central noradrenaline activity may enhance dopamine turnover (Andén and Grabowska, 1976; Hervé et al., 1982). Furthermore, electrophysiological recordings demonstrate that direct stimulation of the locus coeruleus (LC) strongly enhances the activity of dopamine neurons in the VTA, an effect that is abolished by pretreatment with reserpine or an  $\alpha_1$  adrenoceptor antagonist (Grenhoff and Svensson, 1993). The noradrenergic modulation of the dopamine neurons seems to primarily involve regulation of burst firing (Grenhoff and Svensson, 1989; Grenhoff and Svensson, 1993), an effect that has been suggested to be mediated via excitatory  $\alpha_1$  adrenoceptors on dopaminergic cell bodies (Grenhoff et al., 1995; Grenhoff and Svensson, 1993). Central

noradrenaline activity has also been observed to affect extracellular levels of dopamine specifically in the cortical nerve-terminal region (Carboni et al., 1990).

### 1.2.5 DOPAMINE RECEPTORS

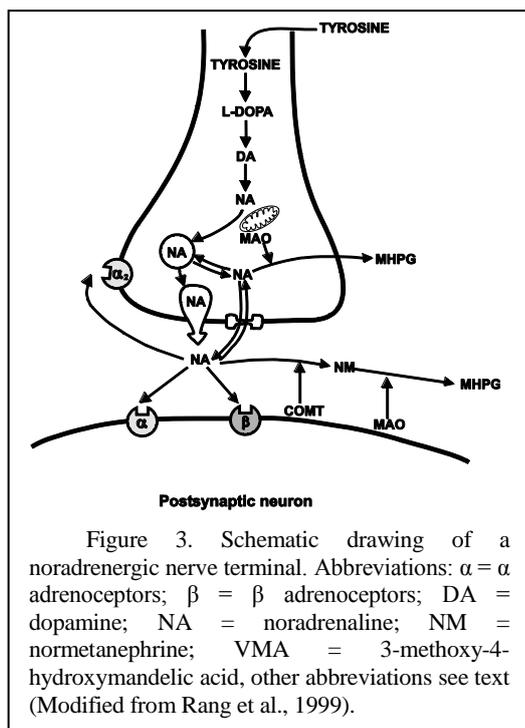
Two types of dopamine receptors, D<sub>1</sub> and D<sub>2</sub>, were originally distinguished on pharmacological and biochemical grounds. Gene cloning revealed further members in these subgroups, the D<sub>1</sub>-like receptors include D<sub>1</sub> and D<sub>5</sub> receptors, while the D<sub>2</sub>-like receptors consists of the D<sub>2</sub>, D<sub>3</sub> and D<sub>4</sub> (see Jaber et al., 1996). All dopamine receptors belong to the family of G-protein coupled transmembrane receptors. Stimulation of D<sub>1</sub>-like receptors for example causes an increase in 3',5'-cyclic adenosine monophosphate (cAMP) levels, whereas stimulation of the D<sub>2</sub>-like receptors generally results in decreased cAMP levels (Spano et al., 1978). Both D<sub>1</sub> and D<sub>2</sub>-like receptors are localized postsynaptically, whereas the presynaptic autoreceptors belong to the D<sub>2</sub> family. Stimulation of dopamine autoreceptors in the somatodendritic region slows the firing rate of dopamine neurons, while stimulation of autoreceptors on the nerve terminals inhibits dopamine synthesis and release. Thus, somatodendritic and nerve terminal autoreceptors work to exert feedback on dopaminergic transmission (see Cooper et al., 2003).

The highest densities of D<sub>1</sub> receptors are found in the NAC, the striatum, the olfactory tubercle and the substantia nigra, but are also found in the thalamus, the hypothalamus and the cerebral cortex (Boyson et al., 1986; Dubois et al., 1986). The D<sub>5</sub> receptors are poorly expressed in the rat brain compared with the D<sub>1</sub> receptors and are restricted to the hippocampus, thalamic and hypothalamic regions (Meador-Woodruff et al., 1992). The highest densities of D<sub>2</sub> receptors have been found in the striatum, the olfactory tubercle, the NAC, the substantia nigra and the VTA. The D<sub>3</sub> receptors are found in high densities in areas such as the NAC, the olfactory tubercle, the islands of Calleja, the cerebellum and the substantia nigra, but also to some extent in the striatum and the VTA (Stanwood et al., 2000). D<sub>4</sub> receptors are more sparsely expressed, and are mainly found in the cerebral cortex and the hippocampus (Van Tol et al., 1991).

## 1.3 THE NORADRENERGIC SYSTEM

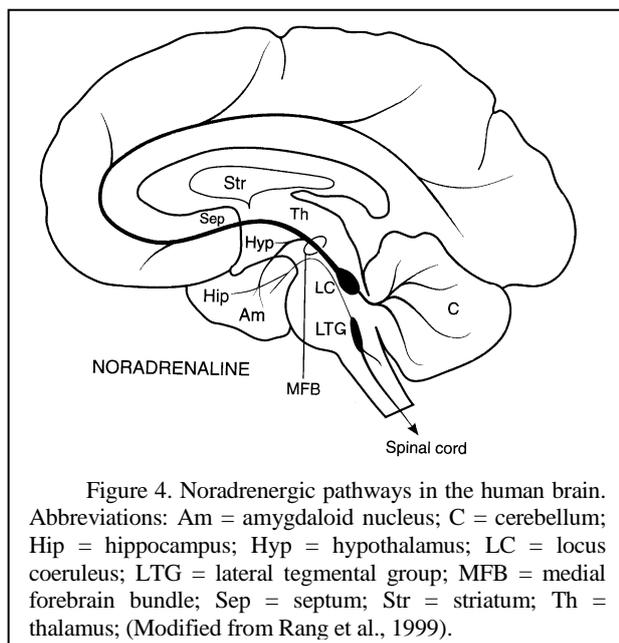
### 1.3.1 NORADRENALINE

The synthesis of noradrenaline follows the same pathway as the synthesis of dopamine. However, in noradrenergic neurons the enzyme dopamine- $\beta$ -hydroxylase hydroxylates dopamine to form noradrenaline. Most of the noradrenaline is stored in vesicles in the nerve terminals, and only little is free in the cytoplasm. Noradrenaline is released, like dopamine, in a calcium-dependent process initiated by nerve impulse activity. The action of released noradrenaline is terminated mainly by reuptake of the transmitter into noradrenergic nerve terminals. Two different reuptake mechanism have been identified, *uptake 1* and *uptake 2*, which differ in location and kinetic properties. Only *uptake 1* is present in neuronal membranes.



Noradrenaline is sequentially metabolized by MAO and COMT to form the major metabolite 3-methoxy-4-hydroxyl-phenethylenglycol (MHPG).

### 1.3.2 THE NORADRENERGIC SYSTEMS IN THE BRAIN



The noradrenergic cells in the brainstem can be divided into two major groups, a lateral tegmental group and the locus coeruleus (LC) group. The lateral tegmental system has its cells of origin distributed diffusely in the medulla and pons and innervates, by its ascending pathways, primarily the hypothalamus and parts of the amygdala, but has also descending projections to the spinal cord. The LC is the main source of the forebrain noradrenergic innervation and project to e.g. the amygdala, the hippocampus as well as most cortical areas.

Two types of adrenoceptors have been described,  $\alpha$  and  $\beta$  adrenoceptors, which are both G-protein coupled receptors. The  $\alpha$  and  $\beta$  adrenoceptors have been further subdivided into  $\alpha_{1A,B,D}$ ,  $\alpha_{2A,B,C}$  and  $\beta_{1,2,3}$  (Bylund, 1988). Stimulation of  $\alpha_1$  adrenoceptors increases levels of inositol triphosphate (IP<sub>3</sub>) and diacyl-glycerol (DAG) through activation of phospholipase C, whereas stimulation of  $\alpha_2$  adrenoceptors inhibits adenylate cyclase activity and decrease cAMP formation. Activation of  $\beta$  adrenoceptors stimulate adenylate cyclase and thus increase the formation of cAMP.

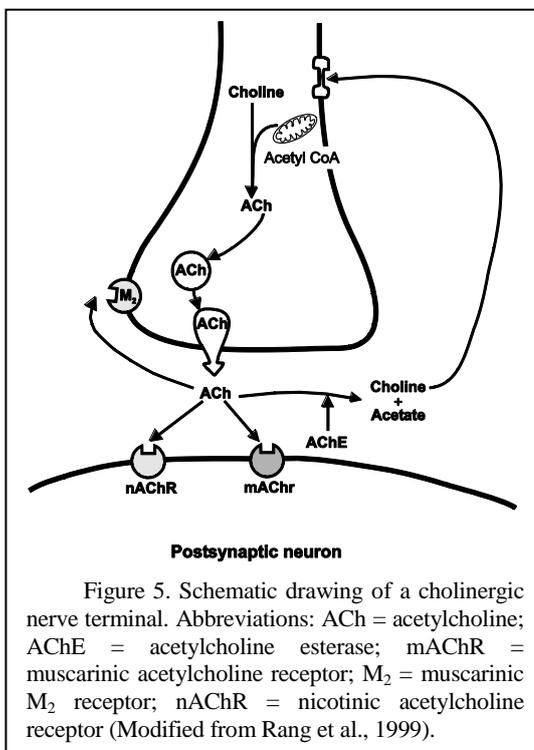
Both  $\alpha_1$  and  $\alpha_2$  adrenoceptors are widely distributed in the rat brain (Nicholas et al., 1996). Originally, it was presumed that the  $\alpha_2$  adrenoceptors were presynaptic and the  $\alpha_1$  adrenoceptors

postsynaptic, but later studies have shown that  $\alpha_2$  adrenoceptors can also be postsynaptically located and  $\alpha_1$  adrenoceptors presynaptically located (see Docherty, 1998).

The level of activation of the LC neurons has been observed to be correlated with vigilance, showing phasic activation responses to environmental sensory stimuli, particularly associated with novelty or fear, but showing low activity in association with behaviors such as grooming, sweet water consumption or sleep (see Svensson and Mathé, 2002). Stimuli of an unfamiliar or threatening kind excite these neurons more effectively than familiar stimuli (see Cooper et al., 2003). Blockade of both  $\alpha_1$  and  $\alpha_2$  adrenoceptors have been suggested to be involved in the mechanisms of action of antipsychotic drugs (Svensson, 2003a).

## 1.4 THE CHOLINERGIC SYSTEM

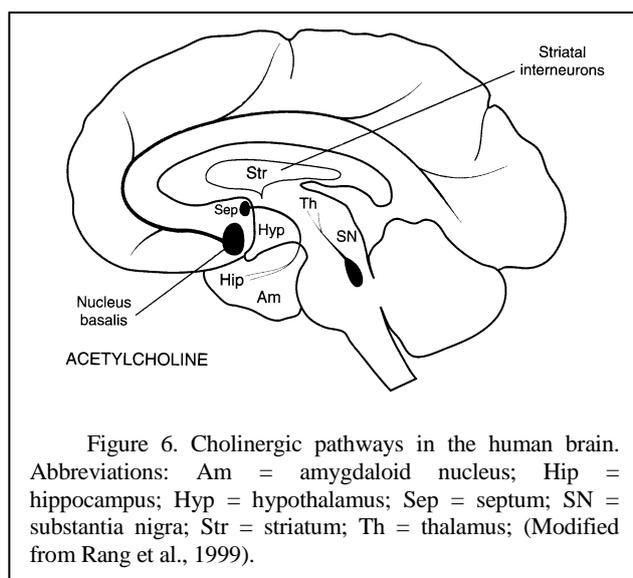
### 1.4.1 ACETYLCHOLINE



ACh is synthesized within the nerve terminal from choline, which is taken up into the nerve terminal by a specific carrier. Free choline within the nerve terminal is acetylated by a cytosolic enzyme, choline acetyltransferase, which transfers the acetyl group from acetyl-CoA. The rate limiting process in ACh synthesis appears to be choline transport. Cholinergic vesicles accumulate ACh actively, by means of a specific carrier and are released in a calcium-dependent process initiated by nerve impulse activity. Cholinergic neurotransmission is terminated when ACh is hydrolyzed by ACh esterase (AChE) which is a very rapid process (Parsons et al., 1993).

### 1.4.2 THE CHOLINERGIC SYSTEMS IN THE BRAIN

ACh is widely distributed in the brain, occurring in all parts of the forebrain (including the cortex), midbrain and brainstem. The two major pathways originate in the magnocellular basal complex (the nucleus basalis and the preoptic magnocellular nucleus), which provide the largest cortical and hippocampal input, and the pedunculopontine - laterodorsal tegmental complex (the pedunculopontine nucleus and the laterodorsal tegmental nucleus), with projections to the thalamic nuclei and the midbrain dopamine neurons (see Gotti and Clementi, 2004). In addition, cholinergic interneurons are found in the striatum (see Cooper et al., 2003). Cholinergic neurotransmission is mediated through muscarinic ACh receptors (mAChRs) as well as through nAChRs. mAChRs have been subclassified as M<sub>1-5</sub> and are all G-protein



coupled receptors and are present in various brain areas. Stimulation of  $M_1$ ,  $M_3$  and  $M_5$  activates phospholipase C, while  $M_2$  and  $M_4$  receptors act by inhibiting adenylate cyclase and thus reduce intracellular cAMP. The nAChRs are ligand-gated cat-ion channels and are widespread in the central nervous system. The nAChRs are pentameric assemblies of different combinations of  $\alpha$  and  $\beta$  subunits, each of which come in several isoforms, The most commonly occurring are the homomeric  $\alpha 7$  and the heteromeric

$\alpha 4\beta 2$  nAChRs (see Gotti and Clementi, 2004). The main functions ascribed to cholinergic pathways are related to arousal, learning and memory, and motor control.

## 1.5 HYPOTHESES OF SCHIZOPHRENIA

### 1.5.1 THE CLASSICAL DOPAMINE HYPOTHESIS OF SCHIZOPHRENIA

In 1952, the antihistaminergic compound chlorpromazine, which was initially used to calm and relax post-operative patients was found to be unique in controlling the symptoms of psychotic patients without excessively sedating them (see Bennett, 1998). The term “neuroleptics” was introduced to denote cataleptic effect of chlorpromazine and reserpine in laboratory animals. In 1958, a new compound, haloperidol was synthesized and was found to be a powerful neuroleptic drug. The so called typical antipsychotic drugs, e.g. haloperidol and chlorpromazine were found to increase the monoamine metabolites, which indicated an increase in monoamine turnover and were thereby suggested to be dopamine antagonists (Carlsson and Lindqvist, 1963). Subsequently, their findings were confirmed by functional studies in the rat (Andén et al., 1970; Andén et al., 1966). Based on these findings, the classical dopamine hypothesis of schizophrenia was formulated suggesting that the disease is a result of a general hyperdopaminergic condition in the brain. Later observations showed that the clinical potency of antipsychotic drugs was positively correlated to their ability to block dopamine  $D_2$  receptors (Creese et al., 1976; Seeman and Lee, 1975). The hypothesis was also supported by studies showing that dopamine releasing drugs, e.g. amphetamine, as well as dopamine  $D_2$  receptor agonists, can aggravate or induce paranoid psychosis (Angrist et al., 1974) and may also worsen schizophrenic symptoms (Angrist and van Kammen, 1984; Snyder et al., 1974). Furthermore, antipsychotic drugs have been shown to label dopamine receptors in patients with schizophrenia (Farde et al., 1992; Farde et al., 1988), and a significant correlation between the degree of  $D_2$  occupancy and effect of antipsychotic drugs in patients has been demonstrated (Nordström et al., 1993)

### 1.5.2 A MODIFIED DOPAMINE HYPOTHESIS OF SCHIZOPHRENIA

Direct evidence for a hyperdopaminergic state in schizophrenia has been complicated to demonstrate, given the difficulty of measuring dopamine transmission in the human brain. However, clinical data have revealed an increased striatal dopamine synthesis (Lindstrom et al., 1999; Reith et al., 1994) as well as increased central dopamine release after amphetamine challenge (Kegeles et al., 2000; Laruelle et al., 1996). These findings suggest that psychotic symptoms may indeed be related to enhanced release of subcortical dopamine. Yet, reduced blood flow in the prefrontal cortex has been shown in some patients with schizophrenia and, specifically, that the blood flow is not enhanced during intellectually challenging tasks. The negative symptoms of schizophrenia have been suggested to be correlated to this hypofrontality (Ingvar, 1987; Ingvar and Franzén, 1974a; Ingvar and Franzén, 1974b; see Knable and Weinberger, 1997). In addition, positive symptoms aggravate following administration of drugs that increase dopaminergic transmission, such as amphetamine, whereas negative symptoms may partly improve (Angrist et al., 1982; van Kammen and Boronow, 1988). Moreover, drugs that block dopamine D<sub>2</sub> receptors or dopamine neuronal storage are able to improve positive symptoms but negative symptoms are less responsive and may even worsen (see Carpenter, 1996). Based on the above findings, the classical dopamine hypothesis has been modified, and a notion of a regional imbalance of central dopamine systems has emerged (Svensson et al., 1995; Weinberger and Lipska, 1995). A modified dopamine hypothesis suggests that hypo- and hyperdopaminergic states may occur in schizophrenic patients in different regions of the brain (Svensson et al., 1995). Thus, whereas a hyperdopaminergic state in subcortical regions may trigger positive symptoms, negative symptoms and cognitive impairment may occur as a result of the hypodopaminergic state in cortical regions.

### 1.5.3 THE GLUTAMATE HYPOTHESIS OF SCHIZOPHRENIA

Administration of the non-competitive NMDA receptor antagonists, such as phencyclidine (PCP) or ketamine induces psychotic states in healthy volunteers almost undistinguishable from schizophrenia (Krystal et al., 1994; Luby et al., 1959; Luby et al., 1962). Moreover, patients with schizophrenia who receive PCP experience a substantial worsening of symptoms (Javitt and Zukin, 1991; Luby et al., 1959). In addition, alterations in expression of mRNA for NMDA receptor subunits in the prefrontal cortex of patients with schizophrenia have been reported (Akbarian et al., 1996). Furthermore, mice expressing only 5% of normal levels of one of the essential NMDA receptor subunits (NR1), display schizophrenia-like behavior abnormalities (Mohn et al., 1999). Based on these observations, schizophrenia has been suggested to be related to a hypoglutamatergic state of the brain.

## 1.6 ANTIPSYCHOTIC DRUGS

The treatment of schizophrenia is focused on the amelioration of symptoms, the prevention of a relapse and social and occupational rehabilitation of the patient. Even though psychosocial treatments, e.g. cognitive, group and family therapies are used in the treatment of

schizophrenia, an important treatment is also pharmacological intervention with antipsychotic drugs.

### 1.6.1 TYPICAL ANTIPSYCHOTIC DRUGS

Although positive symptoms are reduced in a majority of patients treated with typical antipsychotic drugs, these drugs have little or no effect on negative and cognitive symptoms, and may even exacerbate them (Carpenter, 1996). Moreover, since clinically adequate dosage of typical antipsychotic drugs results in a dopamine D<sub>2</sub> receptor occupancy of about 70%, the use of these drugs often induce parkinsonism or other EPS, which become manifest at approximately 80% D<sub>2</sub> occupancy (Farde et al., 1992), which, in turn, may aggravate negative symptoms.

### 1.6.2 ATYPICAL ANTIPSYCHOTIC DRUGS

Clozapine was synthesized in 1958, and was found to exert a powerful antipsychotic effect (see Bennett, 1998). The absence of EPS was noted in animal experiments, as well as in clinical practice, and because of the lack of EPS clozapine was considered an atypical antipsychotic drug. However, clozapine induced many other side effects, such as weight gain and even diabetes, and the most severe side effect agranulocytosis (Idanpaan-Heikkila et al., 1977) led to the withdrawal of clozapine from the market in most countries, but remained in clinical practice in some countries, although only under strict supervision, involving regular haematological monitoring. However, following the discovery that clozapine displays efficacy against treatment-resistant schizophrenia (Kane et al., 1988a; see Lindström, 1988), and thanks to technological advancements in haematological supervision, clozapine was again introduced to the market in 1990. Compared to typical antipsychotic drugs, clozapine was also found to improve negative symptoms, as well as some of the cognitive deficits in patients with schizophrenia (Kane et al., 1988a; see Meltzer and McGurk, 1999).

The unique therapeutic profile of clozapine may be related to its broader spectrum of receptor affinities compared to typical antipsychotic drugs. For example, clozapine acts as an antagonist not only at several dopamine receptors (i.e. D<sub>2</sub>, D<sub>3</sub> and D<sub>4</sub> receptors) but also at, for example, serotonin (5-HT)<sub>2A</sub> and 5-HT<sub>2C</sub> receptors, α<sub>1</sub> and α<sub>2</sub> adrenoceptors, and muscarinic and histaminergic (H<sub>1</sub>) receptors (Ashby and Wang, 1996). Moreover, clozapine may function as a partial agonist at D<sub>1</sub> and 5-HT<sub>1A</sub> receptors (Schotte et al., 1996), raising the possibility that several receptor affinities may contribute to the atypical antipsychotic drug profile (Svensson, 2003b).

Positron emission tomography studies in humans reveal that typical antipsychotics in clinically effective doses yield about 70 to 80% D<sub>2</sub> receptor occupancy in striatal tissue, which approaches the threshold for EPS (Farde et al., 1992). The atypical antipsychotic drug clozapine shows significantly better efficacy than typical antipsychotics including an improved effect on negative symptoms (Kane et al., 1988b), despite a lower D<sub>2</sub> receptor occupancy in brain, which is about 45 to 50% (Farde et al., 1992). Clozapine accordingly has a lower incidence of EPS (Baldessarini and Frankenburg, 1991). The superior therapeutic profile of clozapine has stimulated the development of novel, so called atypical antipsychotic drugs. However, several

of these atypical antipsychotics have other serious side-effects including weight gain, hyperlipidemia and hypertension (see Gardner et al., 2005).

Experimental research has shown that, in contrast to typical antipsychotic drugs, clozapine and most atypicals cause a marked increase in dopamine output in the medial PFC (Kuroki et al., 1999; Moghaddam and Bunney, 1990; Nomikos et al., 1994), an effect of considerable interest because of the role of prefrontal dopamine in cognitive functioning (Arnsten et al., 1994; Brozoski et al., 1979; Sawaguchi and Goldman-Rakic, 1994). Moreover, since the antipsychotic drugs that are most effective against cognitive symptoms also increase extracellular levels of ACh in the cortex, enhancing cholinergic neurotransmission has been suggested to contribute to enhance cognition in schizophrenia (Ichikawa et al., 2002). Since treatment outcome is largely dependent on the degree of cognitive impairment (Green, 1996), it is of great importance that antipsychotic drugs are able to improve not only positive and negative symptoms but also cognitive function. Generally, the commercially available drugs for schizophrenia are insufficient in this regard. Moreover, many patients with schizophrenia are treatment-resistant and hence do not respond to antipsychotic drug treatment. Therefore, the development of more effective and better tolerated antipsychotic drugs is warranted. In addition, developing and evaluating augmentation strategies using combinations of existing antipsychotic drugs and other psychoactive drugs may improve pharmacological treatment of schizophrenia.

### 1.6.3 AUGMENTATION STRATEGIES IN THE TREATMENT – PRECLINICAL ASSESSMENT

Clinical trials have demonstrated that adjuvant treatment with the  $\alpha_2$  adrenoceptor, idazoxan, may augment the clinical efficacy of classical D<sub>2</sub> antagonists (Litman et al., 1993; Litman et al., 1996). In our laboratory, we have previously shown that idazoxan enhances the D<sub>2</sub> antagonist, raclopride-induced suppression of conditioned avoidance response (CAR) as well as the raclopride-induced cortical dopamine output as compared with subcortical regions (Hertel et al., 1999a). Accordingly, the  $\alpha_2$  adrenoceptor blocking effect of clozapine has been hypothesized to be important for its clinical efficacy (Hertel et al., 1999a; Nutt, 1994).

Reboxetine is a selective NA reuptake inhibitor, which is clinically used as an antidepressant drug (Wong et al., 2000). Previously, we have observed that the effects of reboxetine on the mesolimbocortical dopamine system in the rat show several similarities with the effects induced by idazoxan (Grenhoff and Svensson, 1993; Hertel et al., 1999b; Linnér et al., 2001). Based on this background, we decided to evaluate experimentally the potential antipsychotic-like enhancing effect of adjunctive treatment with reboxetine to raclopride, as well as the concomitant EPS liability. In addition, dopamine output in the medial PFC and the NAC was studied.

We also investigated whether adjunctive  $\alpha_2$  adrenoceptor antagonism by means of idazoxan might enhance the antipsychotic-like effects, as well as the regional dopamine output in brain, caused by low doses of a typical (haloperidol) and an atypical (olanzapine) antipsychotic drug, respectively, both possessing low affinity for the  $\alpha_2$  adrenoceptor (see e.g. Bymaster et al., 1999).

Mianserin is a clinically effective antidepressant drug, which acts as an antagonist at 5-HT<sub>2A</sub> and 5-HT<sub>2C</sub> receptors,  $\alpha_2$  and  $\alpha_1$  adrenoceptors, muscarinic and histaminergic (H<sub>1</sub>) receptors (Pinder, 1991). Clinical studies have shown that mianserin enhance the effect of typical antipsychotic drugs, particularly on negative symptoms such as withdrawal retardation, as well as akathisia and some aspects of cognitive dysfunction (Grinshpoon et al., 2000; Iitil et al., 1974; Mizuki et al., 1990; Mizuki et al., 1992; Poyurovsky et al., 2003b; Poyurovsky et al., 1999; Shiloh et al., 2002). This combination generates a clozapine-like clinical effect (Lindström, 2000) as well as a combined receptor binding profile relatively similar to that of clozapine (Bymaster et al., 1996; Pinder, 1991). Therefore, we studied the potential antipsychotic-like effect of adjunctive mianserin to raclopride and also the effects of mianserin and raclopride, alone and in combination on prefrontal and subcortical dopamine output.

AChE inhibitors are used in Alzheimer's disease for symptomatic treatment of cognitive impairment. Small-sized clinical studies indicate that adjunctive use of the AChE inhibitor galantamine, but not donepezil, may improve cognitive impairment in schizophrenia, (Allen and McEvoy, 2002; Bora et al., 2005; Friedman et al., 2002; Rosse and Deutsch, 2002). The beneficial effect of galantamine might be related to the fact that it has two separate mechanisms of action which both enhance cholinergic neurotransmission in the brain. At low doses, it binds allosterically to nAChRs and potentiates their function, and at high doses it acts as a weak AChE inhibitor (Maelicke et al., 2000; Schrattenholz et al., 1996). Previous studies have shown that nicotine by means of activation of nAChRs in the VTA stimulates dopamine cell firing, which in turn causes enhanced dopamine release in terminal areas (Nisell et al., 1994). Consequently, through its action as an allosteric modulator of nAChRs, galantamine may facilitate dopamine release by increasing dopaminergic neuronal activity through potentiation of nAChR function in the VTA. Therefore, using *in vivo* single unit recordings, we investigated the effect of galantamine on VTA dopamine cell firing. Additionally, the effect of galantamine on dopamine output in the prefrontal cortex was examined.

We found that galantamine indeed enhances dopaminergic cell firing *in vivo*, in all probability via allosteric potentiation of nAChRs, an effect that was associated with increased prefrontal dopamine output. This finding was interpreted to support that galantamine might also improve negative and cognitive symptoms in schizophrenia similar to most atypical antipsychotic drugs (Kuroki et al., 1999 c.f. above). In addition, preclinical data indicated that AChE inhibitors may confer some antipsychotic activity even when given alone (Shannon et al., 1999) and two clinical studies had shown that adjunctive AChE inhibitors may improve positive symptoms in schizophrenia (Keefe et al., 2007; Norén et al., 2006). Thus, we decided to investigate the potential antipsychotic-like effect of galantamine and donepezil, respectively, alone and in combination with raclopride. In parallel experiments the EPS liability of these drugs and drug combinations was evaluated.

## 2 SPECIFIC AIMS OF THE STUDY

- To investigate how the antidepressant drug reboxetine might influence the antipsychotic-like effect and EPS liability induced by raclopride, as well as to evaluate the effect of these drugs, alone or in combination, on cortical and subcortical dopamine output.
- To examine experimentally the clinically observed antipsychotic-like effect of the antidepressant drug mianserin when added to raclopride, and also to study how these drugs, alone or in combination, may affect cortical and subcortical dopamine output.
- To study the potential augmentation by the  $\alpha_2$  adrenoceptor antagonist idazoxan of the antipsychotic-like effect and EPS liability of a typical (haloperidol) and an atypical (olanzapine) antipsychotic drug, respectively, and also to study the effects of these drugs, alone or in combination, on cortical and subcortical dopamine output.
- To analyze the effect of galantamine, a combined allosteric potentiator of nAChRs and weak AChE inhibitor, on VTA dopamine cell firing *in vivo* and the mechanisms involved, as well as on cortical dopamine output.
- To evaluate the potential antipsychotic-like effect of galantamine and the selective AChE inhibitor, donepezil, respectively, alone and in combination with raclopride, as well as the potential EPS liability of these drugs and drug combinations.

## 3 MATERIALS AND METHODS

### 3.1 ANIMALS

Male albino rats (Scanbur BK, Sollentuna, Sweden) were used in all studies. Rats from the strain BKI:WR (i.e. Wistar) were used in all microdialysis experiments as well as all behavioral experiments, whereas rats from the strain BKI:SD (i.e. Sprague-Dawley) were used in the electrophysiological experiment in paper IV. The rats were housed, three or four per cage, in the animal facility under standard laboratory conditions with a temperature of  $21.0 \pm 0.4^\circ \text{C}$  with relative humidity of 40-60% in a room with regulated 12-hour light/dark cycle. Food and water was available *ad libitum*. For electrophysiological and microdialysis experiments, the rats were housed on a normal 12-hour light/dark cycle with lights on at 07.00 AM. Animals used in behavioral experiments were housed on a reversed 12-hour light/dark cycle with lights off at 07.00 AM. The rats were kept in their respective light/dark conditions at least five days prior to the experiments to adjust their diurnal rhythm. All experiments were approved by, and conducted in accordance with, the guidelines of the Ethical Committee (Stockholms Norra Försöksdjursetiska Kommittée).

### 3.2 DRUGS

The following drugs were used:

Raclopride: selective dopamine  $D_{2/3}$  receptor antagonist (Astra Zeneca, Södertälje, Sweden), Reboxetine: selective noradrenaline reuptake inhibitor (Pharmacia Corp., Kalamazoo, MI, USA), Mianserin: antagonist at 5-HT<sub>2A/C</sub> receptors,  $\alpha_2$ - and  $\alpha_1$  adrenoceptors, muscarinic and histaminergic ( $H_1$ ) receptors (Sigma-Aldrich, Stockholm, Sweden), Haloperidol: selective dopamine  $D_2$  receptor antagonist (Sigma-Aldrich, Stockholm, Sweden), Olanzapine: dopamine  $D_2$  receptor antagonist, 5-HT<sub>2</sub> receptor antagonist, muscarinic receptor antagonist (Eli Lilly, Indianapolis, IN, USA), Idazoxan: selective  $\alpha_2$  adrenoceptor antagonist (Sigma-Aldrich, Stockholm, Sweden), Galantamine: allosteric potentiator of nAChRs and AChE inhibitor (Janssen Cilag AB, Sollentuna, Sweden), Donepezil: AChE inhibitor (Gift from Janssen Cilag AB, Sollentuna, Sweden), CGP39551: NMDA receptor antagonist (Novartis, Basel, Switzerland), Scopolamine: mAChR antagonist (Sigma-Aldrich, Stockholm, Sweden), Mecamylamine: subtype nonselective nAChR antagonist (Sigma-Aldrich, Stockholm, Sweden), Methyllycaconitine (MLA):  $\alpha_7$ -selective nAChR antagonist (Sigma-Aldrich, Stockholm, Sweden).

Raclopride, reboxetine, mianserin, idazoxan, galantamine, donepezil, CGP39551, scopolamine, mecamylamine and MLA were dissolved in 0.9% saline solution. Haloperidol, olanzapine were dissolved in a minimal amount of glacial acetic acid and subsequently diluted with 5.5 % glucose. In all experiments pH of the drug solutions were adjusted to pH between 5 and 7.3. Reboxetine, mianserin, olanzapine and MLA were administered intraperitoneally, whereas raclopride, haloperidol, idazoxan, galantamine, donepezil, CGP39551, scopolamine and mecamylamine were administered subcutaneously. In paper I, II, IV and V all drugs were

administered in a volume of 1 ml/kg, in paper III all drug were administered in a volume of 2 ml/kg.

### 3.3 CONDITIONED AVOIDANCE RESPONSE

Rats weighing 294 – 522 g were placed in a shuttle-box (530 × 250 × 225 mm) (Kungsbacka Mät- och Reglerteknik, Fjärås, Sweden), divided into two equal compartments by a partition. The rats were free to move from one compartment to the other via an opening (75 × 75 mm) in the partition. Upon presentation of the conditioned stimulus (CS), 80 dB white noise (White Noise Generator, Lafayette 1501, Lafayette, IN, USA), the rat had 10 s to avoid the unconditioned stimulus (USC), an intermittent electric shock in the grid floor of approximately 0.5 mA (intershock interval 2.5 s, shock duration 0.5 s), by moving into the opposite compartment. White noise is a type of noise that is produced by combining equal intensity sounds of all frequencies to form a broad spectrum type of sound. The following behavioral variables were recorded: [1] avoidance (response to CS within 10 s); [2] escape (response to CS + UCS); [3] escape failure (no response to either CS or UCS, if the rat was unable to respond to the UCS within 50 s the trial was terminated). The animals were trained for 5 consecutive days, and were adapted to the shuttlebox 5 minutes before the training session started. Each training session consisted of about 20 trials randomly distributed over 15 min. All subsequent pre-tests and experimental test sessions consisted of about 10 trials randomly distributed over 7.5 min and were performed 20, 90 and 240 minutes after the last drug injection. Experimental manipulations were always preceded by a pre-test. The same animals were tested repeatedly according to a change over design serving as their own controls (Li, 1964). Only rats that performed at least 88% avoidance on the last day of training were included in the experiments. Experimental days were always separated by at least two non-experimental days.

### 3.4 CATALEPSY

Rats weighing 260 – 357 g were placed on an inclined (60°) grid and, excluding the first 30 seconds, the time the rat remained in the same position was measured for a maximum of 2.5 minutes. Experiments were performed 30, 60, 120 and 240 minutes after the last drug injection. The catalepsy was scored from 0 to 5, according to the time (square root transformation) the rat remained immobile: score 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 minutes, i.e. if the rat remained immobile for 0.08 minutes, it was scored as 0, etc. (Ahlenius and Hillegaard, 1986).

### 3.5 MICRODIALYSIS

#### 3.5.1 SURGERY AND MICRODIALYSIS

Rats weighing 230 – 390 g were anaesthetized with sodium pentobarbital (60 mg/kg, intraperitoneally (i.p.), Apoteket, Sweden) (paper I and II) or with a cocktail containing Hypnorm® (Fentanyl 0.315 mg/ml + Fluanison 10 mg/ml, Jansen Cilag) and Dormicum® (Midazolam 5 mg/ml, Roche) diluted in distilled water (1:1:2; 5 mg/kg intraperitoneally (i.p.))

and mounted in a stereotaxic frame (David Kopf, Tujunga, CA, USA) equipped with a heating pad. Additional administrations prior to surgery consisted of atropine (0.05 mg, subcutaneously (s.c.), NM Pharma, Sweden), saline (1 ml, s.c.) and Chloromycetin (1%, eye-salve, Warner Lambert Nordic AB, Sweden) (paper I and II) or Isopto-Plain® (hypromellos 5 mg/ml, eye-drops, Alcon Sverige AB, Sweden) (paper III and IV). Also, after surgery, before suturing the wound, a local anesthetic (Marcain, Astra Zeneca, Sweden) was administered. Concentric dialysis probes were stereotaxically implanted in either in the medial PFC or the NAC. Stereotaxic coordinates were (in mm): anteroposterior (AP) +3.0 or +1.6; mediolateral (ML)  $\pm 0.6$  or  $\pm 1.4$ ; dorsoventral (DV) -5.2 or -8.2, respectively, relative to bregma and dural surface (Paxinos and Watson, 1998). Dialysis occurred through a semipermeable membrane (copolymer of acrylonitrile and sodium methallyl sulfonate) with an inner diameter of 0.24 mm, molecular cutoff at 40 000 Da (AN69 Hospal, Hospal Med, Sweden) and an active surface length 4.0 or 2.25 mm in case of mPFC or NAC, respectively. After surgery the animals were housed individually and given free access to food and water. Dialysis experiments were conducted approximately 48 hours after surgery in freely moving animals in a bright room. The dialysis probe was perfused with a modified Ringer's solution (147 mM sodium chloride, 3.0 mM potassium chloride, 1.3 mM calcium chloride, 1.0 mM magnesium chloride and 1.0 mM sodium phosphate; pH 7.4) at a rate of 2.5  $\mu$ l/minute set by a microinfusion pump (Harvard Apparatus, USA). The dialysate was loaded directly into the sample loop of the injector (Valco Instruments, USA) and was collected over 30 or 15 minutes intervals for medial PFC and NAC experiments, respectively. A personal computer running the turbochrome 4.1 software (Perkin-Elmer, USA) controlled a PE Nelson 900 interface (Perkin-Elmer, USA) to automatically inject samples to the analytical system based on a predetermined program. Upon completion of the experiments, the animals were killed by an overdose of sodium pentobarbital (60 mg/kg, i.p., Apoteket, Sweden) and the brains were taken out and preserved in 25% sucrose, 10% paraformaldehyde solution. Each brain was subsequently sliced, stained with neutral red and examined for probe placement. Only data from experiments where the probe had been correctly placed according to the atlas Paxinos and Watson (1998) was included in the analysis.

### 3.5.2 BIOCHEMICAL ASSAYS

Concentration of dopamine, DOPAC, HVA and 5-hydroxyindole acetic acid (5-HIAA) were determined by high performance liquid chromatography (HPLC) coupled to electrochemical detection. Separation of dopamine, DOPAC, HVA and 5-HIAA was accomplished by reversed phase liquid chromatography through a C-18 column (Supelcosil 3 $\mu$ m, 150  $\times$  4.6 mm), using a 55 mM sodium acetate buffer, pH 3.9-4.0 (adjusted with glacial acetic acid), consisting of 7-11% methanol, 0.1 mM octanesulfonic acid (Phase separations Ltd, UK) and 0.01mM Na<sub>2</sub>EDTA, as mobile phase, delivered at a rate of 0.8 ml/minute. Samples were quantified by sequential oxidation and reduction (+400 mV/-200mV) using a coulometric detector (Coulochem II, model 5200, ESA) feeding output to a two-pen chart recorder (Kipp and Zonen, Netherlands) as well as to a personal computer running the turbochrom 4.1 software.

### 3.5.3 DATA ANALYSIS

Raw data was analyzed using the turbochrom software and was subsequently presented as dialysate concentration over time (fmol/minute), where 100 percent is defined as the average of the last two (medial PFC) or four (NAC) samples preceding drug or vehicle administration. Data were analyzed by one- and two-way (treatment  $\times$  time) analysis of variance (ANOVA) with repeated measures, followed by the Newman-Keuls test for multiple comparisons with a criterion of  $p < 0.05$  to be considered significant. Statistical calculations were computer aided, using the CSS Statistica software.

## 3.6 ELECTROPHYSIOLOGY

### 3.6.1 SURGERY AND EXPERIMENTAL PROCEDURES

Rats weighing 250-300 g were anesthetized with chloral hydrate (400 mg/kg, i.p., KEBO LAB, Sweden) and mounted in a stereotaxic frame (David Kopf, Tujunga, CA, USA). Anesthesia was maintained throughout the experiments with periodical injections of chloral hydrate (approximately 150 mg/kg/hour, i.p.) and body temperature was kept at 37 °C with a rectal thermometer connected to an electrical heating pad. A hole was drilled in the skull above the VTA, ( $3.2 \pm 0.3$  mm anterior of the interaural line and  $0.7 \pm 0.2$  mm lateral to the midline (Paxinos and Watson, 1998). Subsequently the dura mater was cut, and, in order to prevent dehydration, saline (1 ml, s.c.) was administered.

### 3.6.2 EXTRACELLULAR RECORDINGS

Extracellular recording electrodes were pulled in a vertical puller (Narishige, Japan) from glass capillaries (Clark electromedical instruments, UK; outer diameter 1.5 mm, inner diameter 1.17 mm) and filled with 2 % pontamine sky blue in 2 M sodium acetate. The tip of the electrode was broken under microscope, yielding an impedance of 2 – 4 M $\Omega$  at 135 Hz. The electrode was lowered into the brain using a hydraulic microdrive (David Kopf, Tujunga, CA, USA) and a reference electrode was placed in the subcutaneous tissue. Presumed dopamine neurons were found 7.5 – 8.5 mm from the brain surface and were recognized by their characteristic triphasic action potential waveform of more than 2.0 ms duration, basal firing rates of 1 – 10 Hz, and frequent occurrence of burst firing (Wang, 1981). Moreover the “Ungless-filter”, consisting of a tail- or toe-pinch, was applied and only cells that responded with a transient inhibition of firing were included in the study (Ungless et al., 2004). Extracellular electrical activity was amplified, filtered (band pass 0.3 – 3 kHz), discriminated, and monitored on an oscilloscope (TDS 310, Tektronix, Beaverton, OR, USA) and an audiomonitor (Grass, AM8B/C, West Warwick, RI, USA). Discriminated spikes were fed via a CED 1401 interface (Cambridge Electronic Design Ltd, Cambridge, UK) to a computer running CED Spike 2 software. After completion of the experiment, the position of the electrode was marked by iontophoresis of pontamine sky blue into the tissue (5  $\mu$ A for 5 minutes). The rat was killed with an overdose of anesthetic, and the brain was removed and placed in 25 %

sucrose, 10 % paraformaldehyd solution. Sections of the VTA were cut (50  $\mu\text{m}$ ) and stained with neutral red and the recording sites were verified by light microscopy.

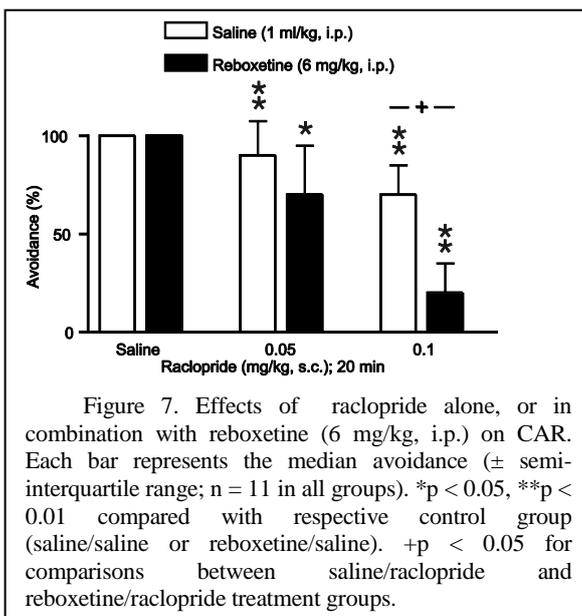
### 3.6.3 DATA ANALYSIS

Dopamine cell firing was analyzed with respect to average firing rate and the percentage of action potentials fired in bursts, calculated over consecutive periods of 500 inter-spike time intervals (ISI). Since the time period during which spikes are analyzed will depend on firing frequency, in cells with firing frequencies lower than 3.0 Hz, consecutive periods of 250 ISI intervals were used to yield better time resolution. In experiments in which only one drug was administered, the last analyzed period before drug injection was compared to the period within 15 min after injection representing the median effect. In experiments in which two drugs were administered, the last period before any drug injection was compared to the median effect observed within 15 min of injection of galantamine (or donepezil). Firing rate data were statistically analyzed with two-way ANOVA followed by Newman–Keul’s test for multiple comparisons or Student’s paired t-test when appropriate. Values for all the individual cells are presented in figures with lines indicating mean  $\pm$  SEM. Analysis of burst firing was performed on the percentage of spikes fired in bursts in the last analyzed period before injection and was compared to the period within 15 min after injection representing the median effect. Burst firing is defined as a series of spikes starting when the interval between two spikes is lower than 80 ms and terminating when the interval exceeds 160 ms (Grace and Bunney, 1984a). Since burst firing was not normally distributed, the nonparametric Kruskal–Wallis ANOVA or the Mann–Whitney U-test was used to analyze differences between treatments and Wilcoxon matched pairs signed ranks test was used to evaluate within effects of the different treatments. Data are presented in figures as absolute pre- and postinjection values for all the individual cells and the median across cells is indicated with a horizontal line.

## 4 RESULTS AND DISCUSSION

### 4.1 EFFECTS OF ADJUNCTIVE REBOXETINE TREATMENT ON RACLOPRIDE INDUCED CHANGES IN BEHAVIOR AND CENTRAL DOPAMINE OUTPUT (PAPER I)

Among other properties, clozapine displays considerable affinity for  $\alpha_2$  adrenoceptors (see Ashby and Wang, 1996), and we have previously reported that adjunctive treatment with the  $\alpha_2$  adrenoceptor antagonist idazoxan significantly augments the suppression of CAR induced by the selective  $D_{2/3}$  receptor antagonist raclopride without increasing the raclopride-induced catalepsy (Hertel et al., 1999a). In addition, central dopamine output, specifically in the medial PFC, was markedly enhanced. Since we had previously observed that the effects of the noradrenaline reuptake inhibitor reboxetine on the mesolimbocortical dopamine system in the rat show several similarities to those induced by idazoxan (cf above, Introduction), the potential antipsychotic-like effect of addition of reboxetine to raclopride was experimentally evaluated in the CAR test. EPS liability was also assessed by means of the catalepsy test.

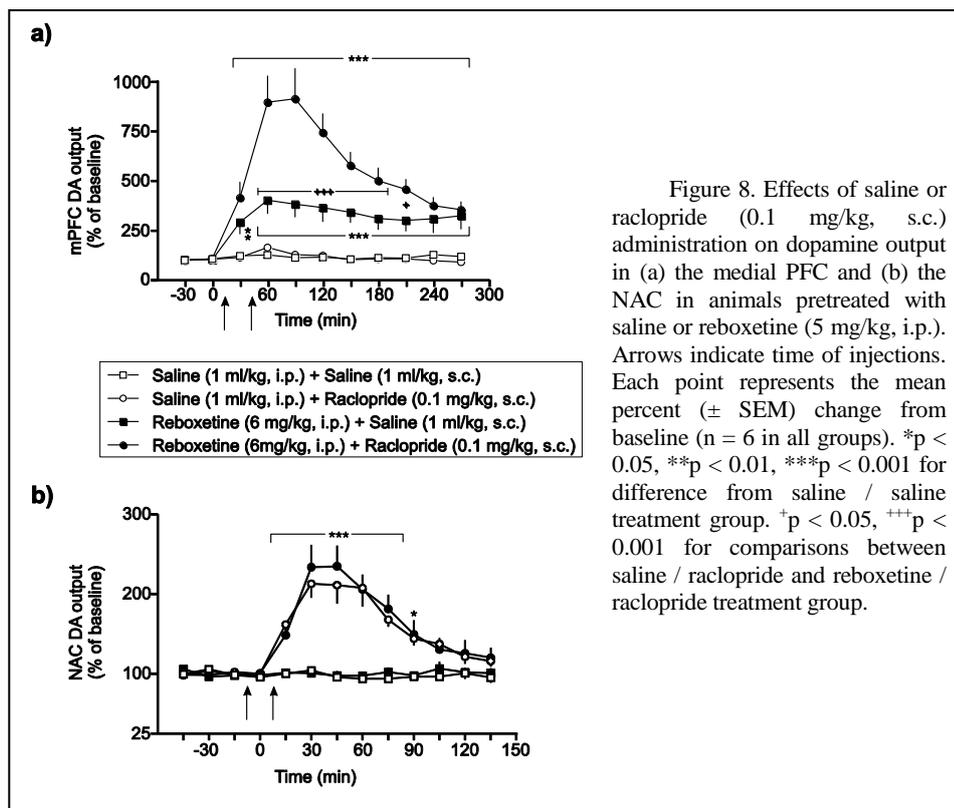


The suppressant effect of raclopride in the CAR test, when given in a dose resulting in approximately 65%  $D_2$  receptor occupancy in the rat (0.1 mg/kg, s.c.) (Wadenberg et al., 2000b), was significantly augmented by additional treatment with reboxetine (6.0 mg/kg, i.p.) (figure 7), without any concomitant effect on catalepsy scores. Treatment with reboxetine alone had no effect on either avoidance behavior in the CAR test or on catalepsy scores. Thus, our results indicate that the clinical efficacy of a typical antipsychotic drug may be augmented by adjunctive treatment with reboxetine, yet

without inducing EPS. This conclusion derives further support from previous studies showing that adjunctive imipramine (Siris et al., 1990) or desipramine (Hogarty et al., 1995), another antidepressant drug that act primarily by blocking noradrenaline reuptake, may also enhance the effect of antipsychotic drugs. Recent studies suggest that the beneficial effect of adjunctive reboxetine treatment is not limited to typical antipsychotics but may also be obtained with atypical antipsychotic drugs (Raedler et al., 2004). In addition, adjunctive reboxetine has been shown to reduce weight gain in olanzapine treated patients (Poyurovsky et al., 2003a).

The effects of reboxetine alone and in combination with raclopride on dopamine output in the medial PFC and the NAC, respectively, was also evaluated. Raclopride did not affect dopamine output in the medial PFC, whereas, reboxetine significantly increased it. The combination of raclopride and reboxetine caused a massive increase of dopamine output in the

medial PFC. In contrast, in the NAC, the addition of reboxetine to raclopride did not affect the raclopride-induced dopamine output (figure 8).



We thus observed an increase in cortical dopamine output without any change in subcortical dopamine outflow (figure 8). Hence, by adding reboxetine to a typical antipsychotic drug, the biochemical profile in this regard resembles that of most atypical antipsychotic drugs (see Kuroki et al., 1999; Westerink et al., 2001). Similar effects have also been demonstrated with other noradrenaline reuptake inhibitors, such as atomoxetine (Bymaster et al., 2002; Kratochvil et al., 2003) and desipramine (Carboni et al., 1990; Yamamoto and Novotney, 1998).

The medial PFC is an area with high expression of noradrenaline transporters but a low expression of dopamine transporters. This is important since a significant proportion of dopamine in the medial PFC may be removed from the extracellular space by the noradrenaline transporter (Carboni et al., 1990; Gresch et al., 1995; Yamamoto and Novotney, 1998), and the enhancing effect of reboxetine on dopamine output in the medial PFC is probably to a large extent related to blockade of this mechanism.

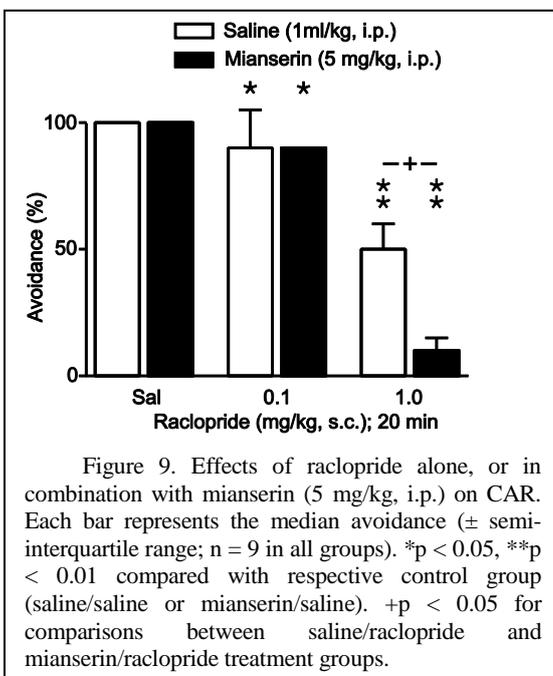
Interestingly, the atypical antipsychotic drug quetiapine has been found to increase both noradrenaline and dopamine output in the PFC (Pira et al., 2004), and recently the major active metabolite of quetiapine in humans, nor-quetiapine, has been shown to potently inhibit the noradrenaline transporter (Goldstein et al., 2007; Nyberg et al., 2007). In fact, *in vitro* studies also show that both olanzapine and clozapine may to some extent inhibit the noradrenaline transporter at clinically therapeutic plasma concentrations (Yoshimura et al., 2005; Yoshimura et al., 2000). This inhibitory effect of some of the antipsychotic drugs or their metabolites, such

as nor-quetiapine, on the noradrenaline transporter may clearly contribute to the effect of such drugs in schizoaffective disorders.

#### 4.2 EFFECTS OF ADJUNCTIVE MIANSERIN TREATMENT ON RACLOPRIDE INDUCED CHANGES IN BEHAVIOR AND CENTRAL DOPAMINE OUTPUT (PAPER II)

Clinically, mianserin had been found to augment the therapeutic effect of typical antipsychotic drugs (cf. Introduction). Moreover, adjunctive treatment with mianserin, which is an antagonist at  $\alpha_2$  and  $\alpha_1$  adrenoceptors, 5-HT<sub>2A</sub> and 5-HT<sub>2C</sub> receptors, muscarinic and histaminergic (H<sub>1</sub>) receptors (Pinder, 1991), to the selective dopamine D<sub>2/3</sub> receptor antagonist, raclopride, should generate a combined receptor binding profile resembling that of clozapine (Bymaster et al., 1996; cf Pinder, 1991).

The potential antipsychotic-like activity of the addition of mianserin to raclopride was experimentally evaluated in the CAR test. In addition, the EPS liability was assessed by means

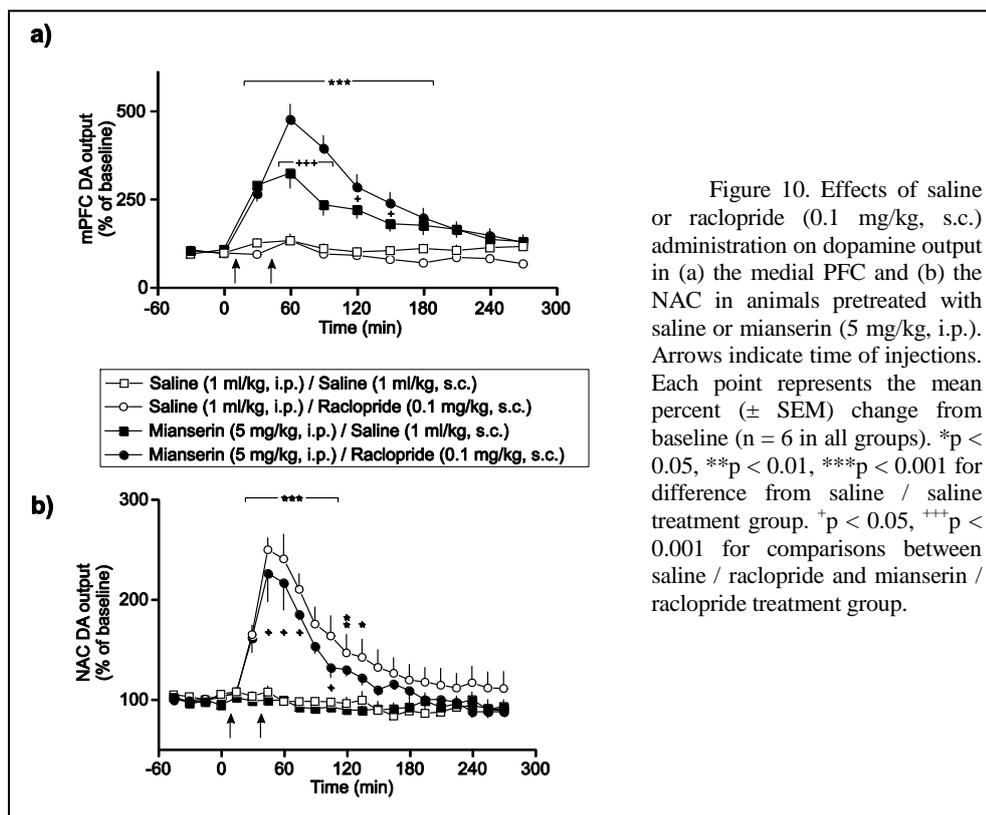


of the catalepsy test. Using *in vivo* microdialysis, the effects of mianserin, alone and in combination with raclopride, on dopamine output in the medial PFC and the NAC, respectively, were also studied.

The addition of mianserin (5 mg/kg, i.p.) significantly enhanced the raclopride (0.1 mg/kg, s.c.)-induced suppression of CAR (figure 9). The addition of mianserin to raclopride did not significantly affect catalepsy scores, although there was, in fact, a tendency for mianserin to reverse the raclopride (1.0 mg/kg, s.c.)-induced catalepsy. Treatment with mianserin alone, had no effect in the CAR test or on catalepsy scores. These experimental data, which thus

parallel the previous clinical results, generally support the validity of the CAR model for use in augmentation studies.

Since mianserin possesses affinities for several monoaminergic receptors, one or several of these might be involved in the antipsychotic-like effect. Treatments with the 5-HT<sub>2A/C</sub> receptor antagonist ritanserin (Wadenberg et al., 1996), the selective 5-HT<sub>2A</sub> receptor antagonist M100907 (Wadenberg et al., 1998), the  $\alpha_2$  adrenoceptor antagonist idazoxan (Hertel et al., 1999a) as well as the  $\alpha_1$  adrenoceptor antagonist prazosin (Wadenberg et al., 2000a) have all been found to potentiate the suppression of CAR by raclopride, hence, blockage of one or several of these receptors by mianserin may be causally related to the enhanced antipsychotic-like effect.



The addition of mianserin (5 mg/kg, i.p.) to raclopride (0.1 mg/kg, s.c.) was found to induce a preferential increase in prefrontal dopamine output, as compared with the NAC (figure 10). It has previously been reported that both the  $\alpha_1$  adrenoceptor antagonist prazosin and the 5-HT<sub>2A</sub> antagonist M100907 inhibit the D<sub>2</sub> receptor antagonist induced increase in accumbal dopamine release (Andersson et al., 1995; Liegeois et al., 2002). In contrast, 5-HT<sub>2C</sub> receptor blockage activates both cortical and subcortical dopamine projections (Di Matteo et al., 2000; Gobert et al., 2000), suggesting that the preferential enhancement of prefrontal dopamine efflux by atypical antipsychotic drugs may be associated with blockade of  $\alpha_2$  adrenoceptors or 5-HT<sub>2A/C</sub> receptors. However, since blockade of  $\alpha_1$  adrenoceptors has not been associated with increased prefrontal dopamine outflow (Weikop et al., 2004) this receptor affinity of mianserin may not necessarily contribute to its effect on negative symptoms (see Eltayb et al., 2005).

Our results are consonant with several clinical studies which report that mianserin may enhance the clinical effect of typical antipsychotic drugs (Grinshpoon et al., 2000; Hayashi et al., 1997; Iitil et al., 1974; Mizuki et al., 1990; Mizuki et al., 1992; Shiloh et al., 2002), particularly on negative symptoms such as withdrawal retardation. In addition, some aspects of cognitive dysfunction were improved (Poyurovsky et al., 2003b). Mirtazapine, an antidepressant drug with a binding profile rather similar to that of mianserin, has also been found to improve negative symptoms in schizophrenia when added to haloperidol, risperidone, olanzapine and even clozapine (Berk et al., 2001; Zoccali et al., 2004; Zoccali et al., 2003).

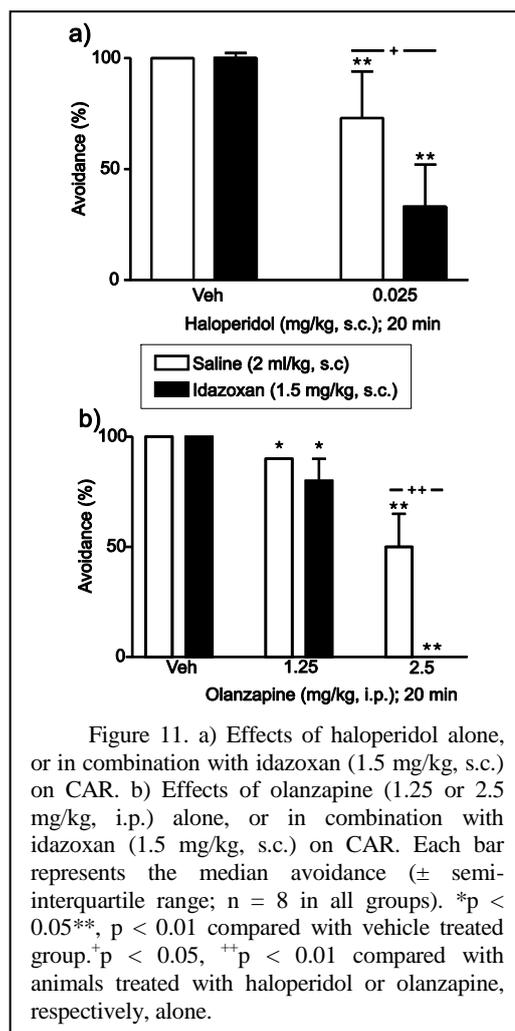
Incidence rates for acute akathisia with typical neuroleptics vary from 8% to 76%, with 20% to 30% being a conservative estimate (Sachdev, 1995). Although the atypical antipsychotics as a group represent an improvement in terms of tolerability, they are still associated with some risk of akathisia, with incidence rates up to 18% (Halliday et al., 2002). Importantly, both mianserin and mirtazapine decrease neuroleptic-induced akathisia

(Miodownik et al., 2006; Poyurovsky et al., 1999; Poyurovsky and Weizman, 1997; Ranjan et al., 2006; Stryjer et al., 2004). However, although adjunctive treatment with mianserin may reduce akathisia, several cases of agranulocytosis have also been reported (Lucht et al., 2000; van der Klauw et al., 1998).

In summary, several pharmacological properties of mianserin, in particular blockade of 5-HT<sub>2A/C</sub> receptors and  $\alpha_2$  as well as  $\alpha_1$  adrenoceptors may contribute to augment the effect of typical antipsychotic drugs in schizophrenia. This improvement appears to include not only an effect on negative symptoms, but also a reduction of dysphoria, anxiety, akathisia, and potentially also cognitive dysfunction and, generally, the overall effect seems to mimic the clinical profile of clozapine.

#### 4.3 EFFECTS OF ADJUNCTIVE IDAZOXAN TREATMENT ON CHANGES IN BEHAVIOR AND CENTRAL DOPAMINE OUTPUT INDUCED BY HALOPERIDOL OR OLANZAPINE (PAPER III)

Clozapine is, as previously mentioned, a potent antagonist at  $\alpha_2$  adrenoceptors (Ashby and Wang, 1996), while most other atypical antipsychotic drugs, with the exception of risperidone, have low affinity for this receptor. Clinical studies had previously shown that adjunctive treatment with the selective  $\alpha_2$  adrenoceptor antagonist idazoxan significantly may augment the

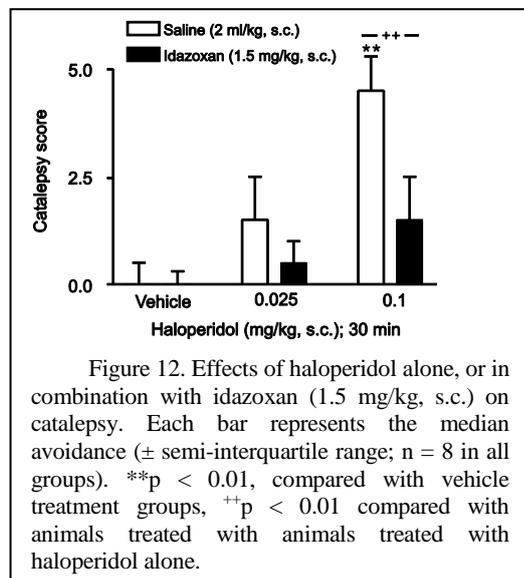


efficacy of a typical antipsychotic drug, fluphenazine, in treatment-resistant schizophrenic patients with an effect size similar to that of clozapine (cf. Introduction). We have previously shown that addition of idazoxan dramatically can augment the antipsychotic-like effect of low doses of the selective D<sub>2/3</sub> receptor antagonist raclopride (Hertel et al., 1999a).

Using the CAR test, we here investigated whether adjunctive idazoxan treatment might also enhance the effects of low doses of a typical (haloperidol) and an atypical (olanzapine) antipsychotic drug, respectively, both possessing low affinity for the  $\alpha_2$  adrenoceptor (see e.g. Bymaster et al., 1999). The EPS liability was also assessed by means of the catalepsy test.

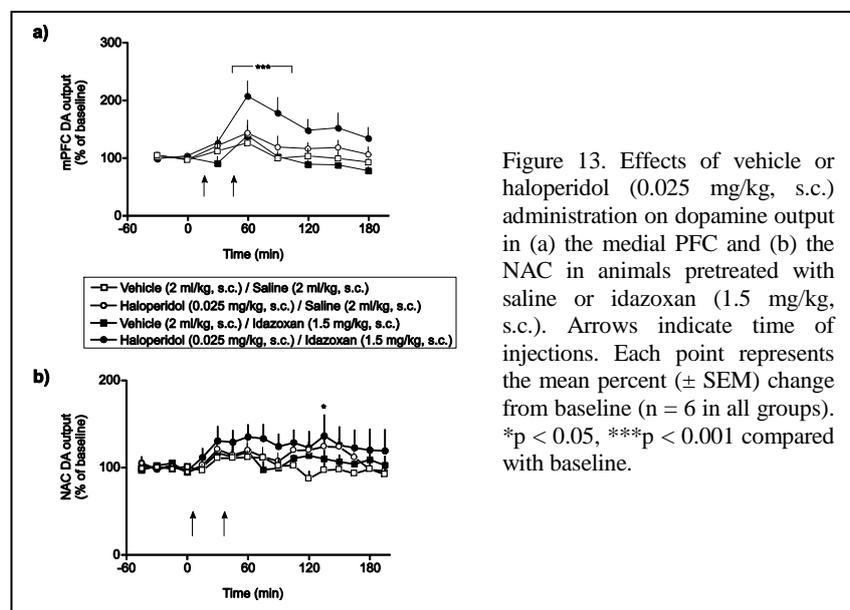
While idazoxan (1.5 mg/kg, s.c.), when administered alone, did not suppress CAR, it augmented the suppression of CAR induced by a very low dose (0.025 mg/kg, s.c.) of haloperidol (figure 11a). Moreover, the addition of idazoxan significantly augmented the olanzapine (2.5 mg/kg, i.p.)-induced suppression of CAR (figure 11b).

Thus, the addition of the  $\alpha_2$  adrenoceptor antagonist idazoxan may enhance the antipsychotic-like effect of both a typical and an atypical antipsychotic drug. These data also support the notion that  $\alpha_2$  adrenoceptor antagonism may be a critical determinant of the superior efficacy of clozapine in schizophrenia.



Interestingly, while idazoxan (1.5 mg/kg, s.c.) alone did not affect catalepsy scores the addition of idazoxan was found to significantly reverse catalepsy induced by 0.1 mg/kg of haloperidol (figure 12). Previously, addition of  $\alpha_2$  adrenoceptor antagonists to neuroleptics has been found to reduce EPS liability (see Hertel et al., 1999a; Kalkman et al., 1998). Olanzapine, neither alone nor in combination with idazoxan induced any catalepsy.

Using microdialysis, the combined treatment of haloperidol (0.025 mg/kg, s.c.) and idazoxan (1.5 mg/kg, s.c.) was found to increase dopamine output in the medial PFC (figure 13a), whereas neither of the drugs had any effect when administered alone. In addition, the combined treatment of idazoxan and haloperidol produced a slight increase in dopamine output in the NAC that was significant only at one time-point (figure 13b).



In contrast to haloperidol, olanzapine increased dopamine output in both the medial PFC and the NAC. The addition of idazoxan (1.5 mg/kg, s.c.) significantly augmented the olanzapine (2.5 mg/kg, i.p.)- induced dopamine output in the medial PFC (figure 14a), whereas the addition of idazoxan did not significantly affect the olanzapine-induced dopamine output in

the NAC (figure 14b). Thus, the addition of idazoxan to either a low dose of haloperidol or olanzapine preferentially increases prefrontal dopamine output to a level approaching that of clozapine.

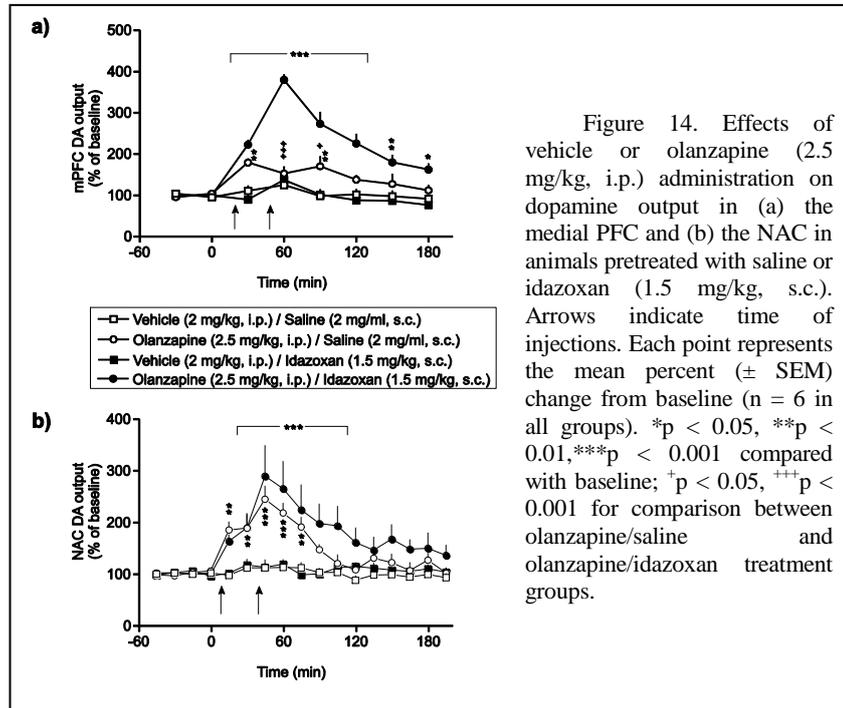


Figure 14. Effects of vehicle or olanzapine (2.5 mg/kg, i.p.) administration on dopamine output in (a) the medial PFC and (b) the NAC in animals pretreated with saline or idazoxan (1.5 mg/kg, s.c.). Arrows indicate time of injections. Each point represents the mean percent ( $\pm$  SEM) change from baseline ( $n = 6$  in all groups). \* $p < 0.05$ , \*\* $p < 0.01$ , \*\*\* $p < 0.001$  compared with baseline; + $p < 0.05$ , +++ $p < 0.001$  for comparison between olanzapine/saline and olanzapine/idazoxan treatment groups.

In summary, these results propose that adjunctive idazoxan when used together with antipsychotic drugs lacking appreciable affinity for the  $\alpha_2$  adrenoceptor may provide increased antipsychotic efficacy at lower doses, implying less  $D_2$  occupancy and EPS liability as well other dose-related side effects. Through this work we also verify and extend the previous results of Hertel et al. (1999a), and provide further experimental support for the significance of  $\alpha_2$  adrenoceptor antagonism for the efficacy of antipsychotic drugs.

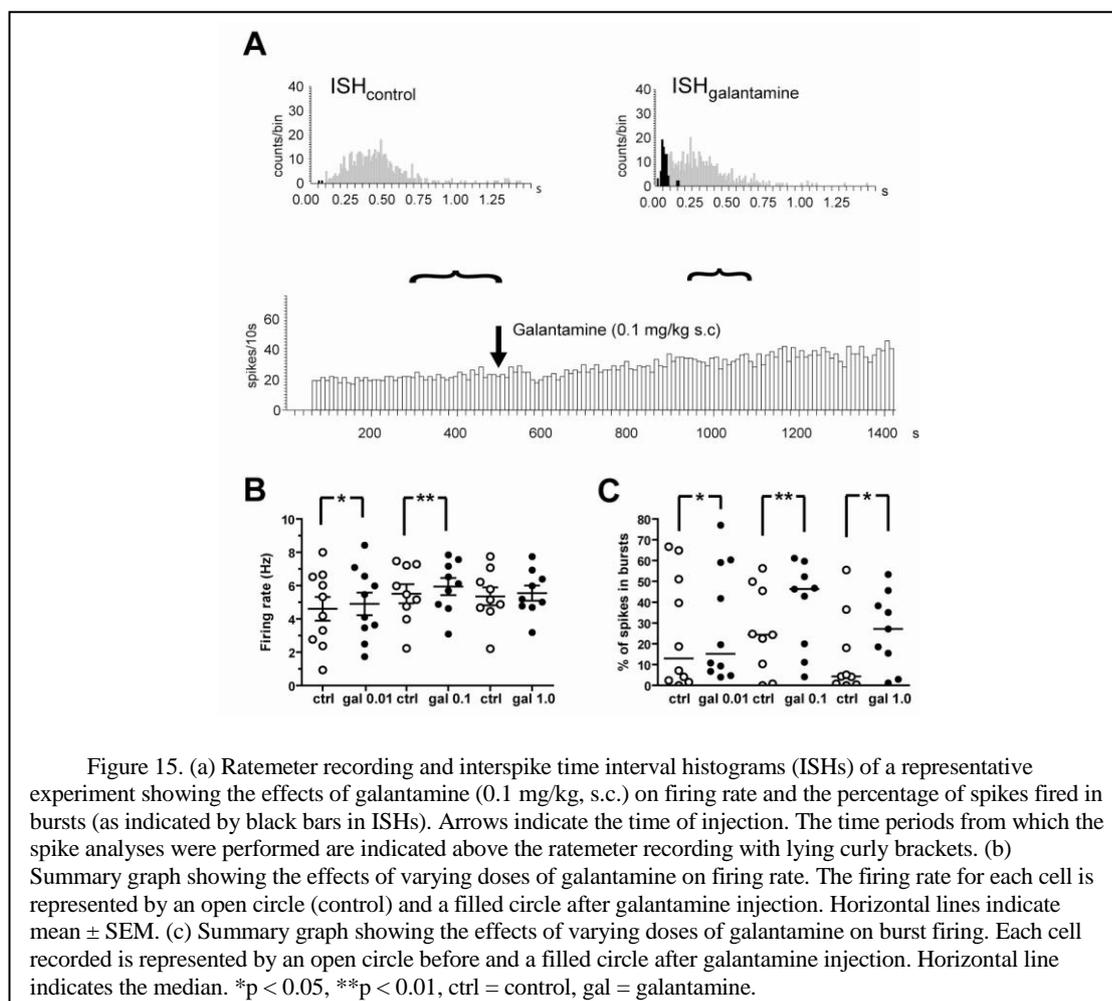
Using the eight-arm radial maze test, we recently showed that the combination of idazoxan and the  $D_{2/3}$  receptor antagonist raclopride completely reversed the disruption of working memory performance induced by the selective NMDA receptor antagonist MK-801 in rats (Marcus et al., 2005). Against this background our present results, showing an increased prefrontal dopamine output by addition of idazoxan to both haloperidol and olanzapine suggest that adjunctive  $\alpha_2$  adrenoceptor blockage may confer a pro-cognitive profile to these antipsychotic drugs.

#### 4.4 EFFECTS OF GALANTAMINE ON DOPAMINERGIC NEURONAL ACTIVITY *IN VIVO* AND DOPAMINE OUTPUT IN THE MEDIAL PFC (PAPER IV)

AChE inhibitors are currently used for symptomatic treatment in Alzheimer's disease. Several small clinical studies indicate that the AChE inhibitor galantamine, but not necessarily donepezil, may improve cognitive function in schizophrenia (Allen and McEvoy, 2002; Bora et al., 2005; Friedman et al., 2002; Rosse and Deutsch, 2002). The effect of galantamine might be explained by a dual mechanism of action. At low doses it allosterically potentiates nAChRs and at higher doses it also acts as a weak inhibitor of AChE (Maelicke et al., 2000; Schratzenholz et

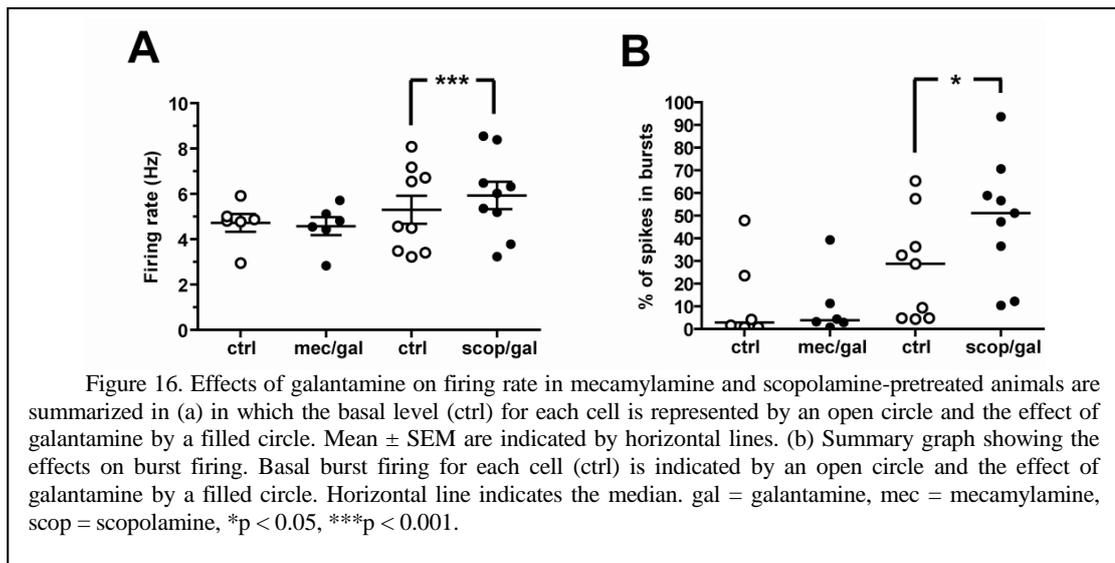
al., 1996). Previous studies have shown that nicotine by means of activation of nAChRs in the VTA stimulates dopamine cell firing, which in turn causes enhanced dopamine release in terminal areas (Nisell et al., 1994). Thus, through its action as an allosteric potentiator of nAChRs, galantamine may facilitate dopamine neurotransmission by a similar mechanism and thereby improve cognitive function. Therefore, using *in vivo* single unit recording techniques, we analyzed the effect of galantamine on dopamine cell firing as well as the mechanisms involved. Additionally, the effect of galantamine on dopamine output in the medial PFC was examined.

Galantamine (0.01 and 0.1 mg/kg, s.c) was found to increase dopamine cell firing rate, whereas the highest dose tested (1.0 mg/kg, s.c.) had no effect. All three doses (0.01, 0.1 and 1.0 mg/kg, s.c.) increased burst firing of VTA dopamine neurons (figure 15).



The stimulatory effect of galantamine on dopaminergic cell firing could be due to either allosteric potentiation of nAChRs, inhibition of AChE or to both mechanisms. Moreover, if the effects were to be caused by inhibition of AChE, which increases the levels of ACh, the effect could be mediated by nAChRs and/or mAChRs. Therefore, the effect of galantamine was tested in the presence of an antagonist at nAChRs and mAChRs, respectively. The nAChR antagonist mecamylamine inhibited the increase in firing rate and burst firing induced by galantamine, but the mAChR antagonist scopolamine had no effect in this regard. These data propose that the

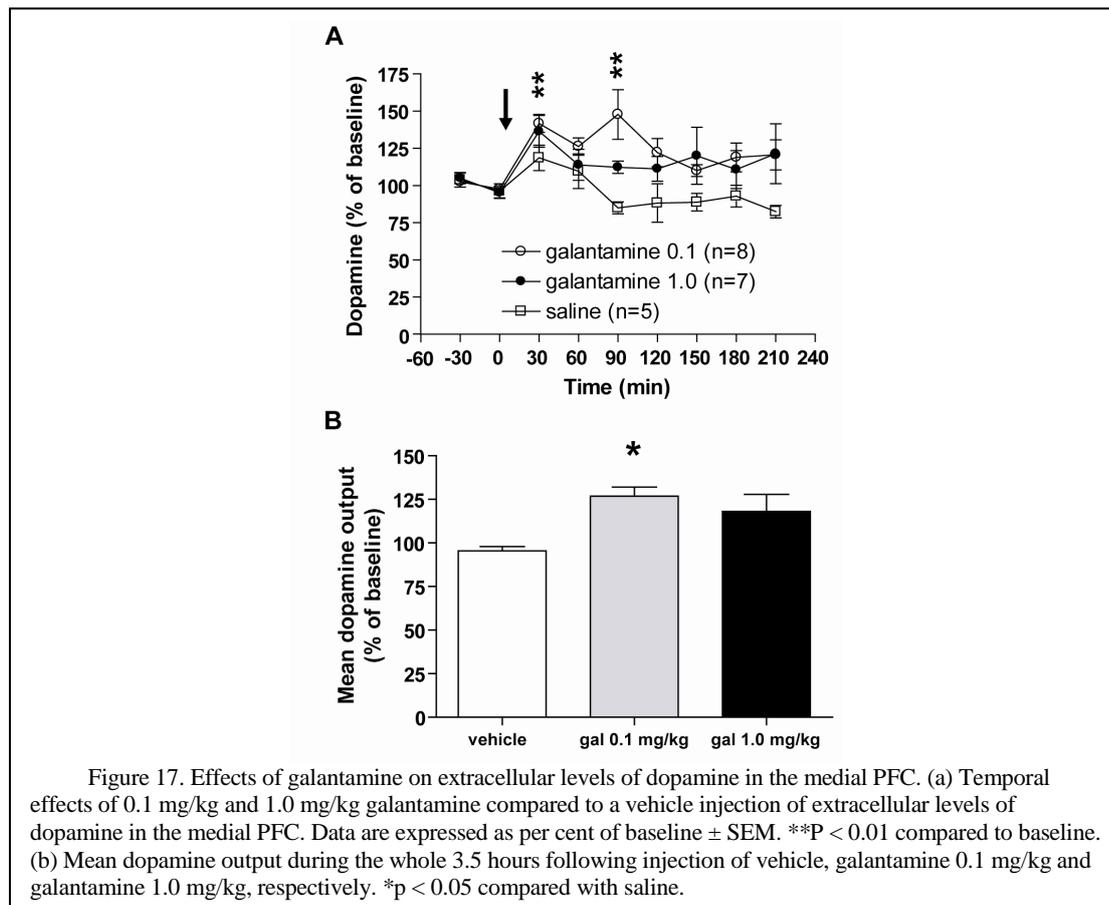
activation of dopamine cell firing of low doses of galantamine is indeed mediated through nAChRs (figure 16).



This conclusion was further supported by the fact that the effect of galantamine could not be mimicked by a different and potent AChE inhibitor, donepezil, which lacks any potentiating effect on nAChRs indicating that the effect of galantamine is not a result of AChE inhibition. In fact, donepezil (5.0 mg/kg) even depressed the firing rate of dopaminergic cells in the VTA, an effect that was abolished when the mAChR antagonist scopolamine was given before donepezil, suggesting that the inhibitory effect of donepezil is mediated through mAChRs.

$\alpha 7$  nAChRs in the VTA have been shown to be involved in nicotine-induced burst firing (Schilström et al., 2003). Previous data demonstrate that presynaptic  $\alpha 7$ -containing nAChRs in the VTA can enhance glutamate release and activate NMDA receptors on dopamine cells (Mansvelder and McGehee, 2000; Schilström et al., 2000; Schilström et al., 1998a; Schilström et al., 1998b). Therefore, with the purpose to investigate the putative involvement of  $\alpha 7$  nAChRs and NMDA receptors, respectively, the effect of galantamine was tested in rats pretreated with the  $\alpha 7$  nAChR antagonist methyllycaconitine (MLA, 6.0 mg/kg, i.p.) or the NMDA receptor antagonist CGP39551 (2.5 mg/kg, s.c.). In MLA-pretreated animals, galantamine had no significant effect on either firing rate or burst firing. Analogously, neither firing rate nor burst firing was affected following galantamine administration to rats pretreated with CGP39551. These pharmacological results are clearly consonant with the notion that galantamine activates dopamine cell firing through allosteric potentiation of nAChRs. This conclusion is based on the observations that the effect of galantamine was not dose-dependent, that it was antagonized by the nAChR antagonist mecamylamine, but not by the mAChR antagonist scopolamine, and that it was not mimicked by the selective AChE inhibitor donepezil. Our results also propose that the effect of galantamine involves an  $\alpha 7$  nAChR-mediated presynaptic facilitation of glutamate release that activates NMDA receptors, since it was prevented both by the  $\alpha 7$  nAChR antagonist MLA and the NMDA receptor antagonist CGP39551. Galantamine has been shown to indirectly increase NMDA currents (Moriguchi et al., 2004) opening the possibility that the effect of galantamine on dopamine cell firing is due to potentiation of NMDA receptors rather than nAChRs. However, since  $\alpha 7$  nAChRs are mainly

localized presynaptically and we were able to block the effects of galantamine with the  $\alpha 7$  nAChR antagonist MLA, the effect of galantamine in all probability involves allosteric potentiation of presynaptic  $\alpha 7$  nAChRs.



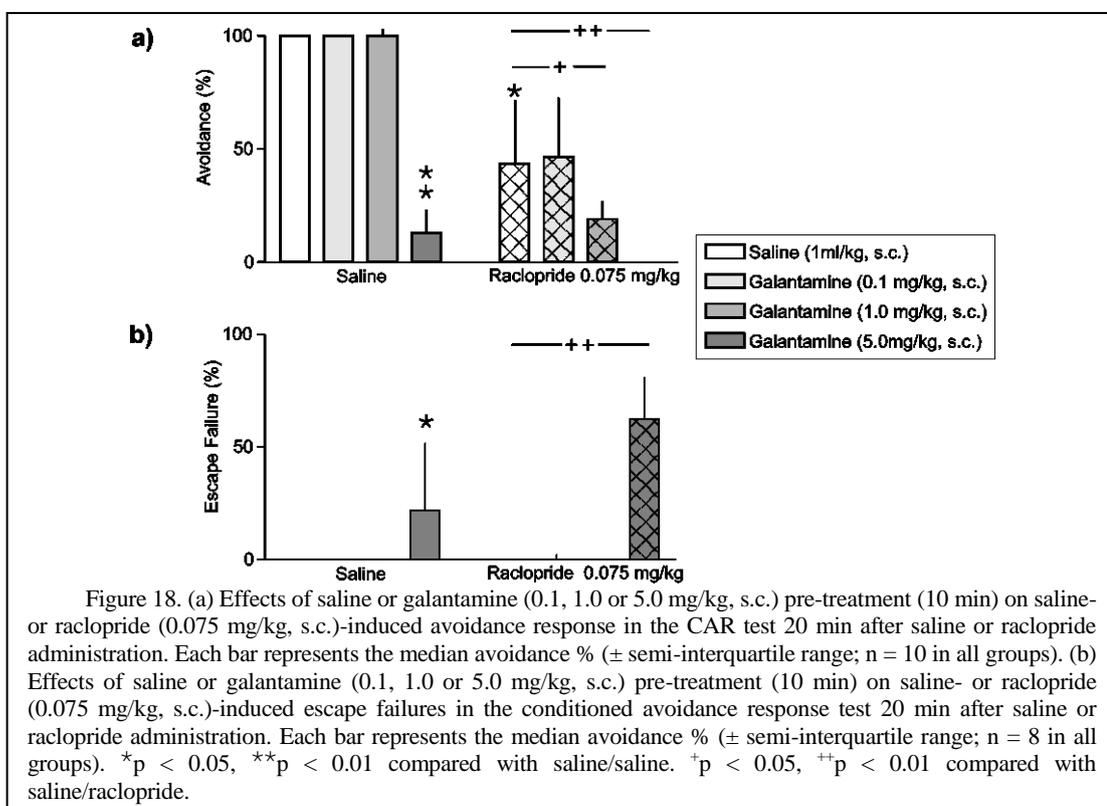
By means of microdialysis, the effect of galantamine (0.1 and 1.0 mg/kg, s.c.) on extracellular dopamine levels in the medial PFC was evaluated. Here, we observed that only the lower dose of galantamine significantly increased dopamine levels in the medial PFC (figure 17a). In addition, only the lower dose significantly increased the mean dopamine output (figure 17b), indicating that this effect rather is due to allosteric potentiation of nAChRs than to AChE inhibition. As mentioned previously, increased dopamine output in the medial PFC is an effect obtained with most atypical but not typical antipsychotic drugs (Kuroki et al., 1999), and, moreover, this effect is thought to contribute to enhanced efficacy in schizophrenia (Eltayb et al., 2005; Hertel et al., 1999a; Kuroki et al., 1999). These results thus provide principle support for the potential utility of galantamine to enhance the antipsychotic effect of typical  $D_2$  antagonists.

#### 4.5 EFFECTS OF ADJUNCTIVE TREATMENT WITH GALANTAMINE AND DONEPEZIL, RESPECTIVELY, WHEN ADDED TO RACLOPRIDE IN THE CAR AND CATALEPSY TESTS (PAPER V)

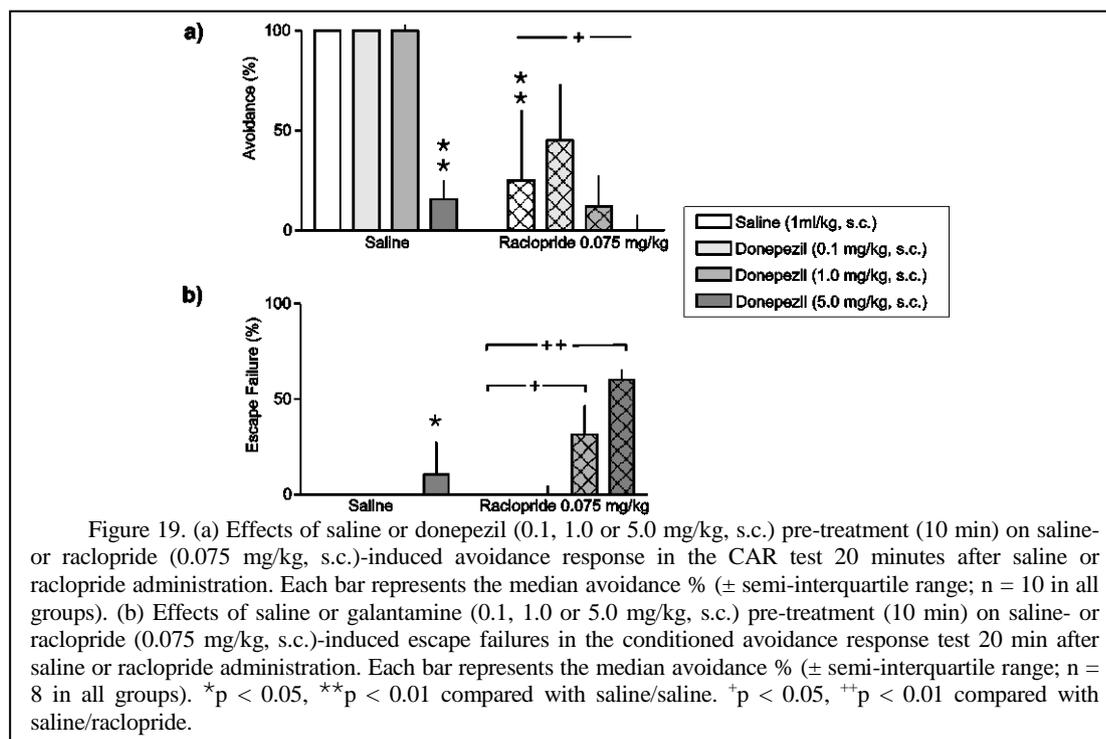
Based on our previous result that galantamine increases dopamine output in the medial PFC, an effect that in itself may be related to antipsychotic efficacy (see above) and that some

evidence has suggested a potential antipsychotic activity of AChE inhibitors, we here evaluated, experimentally, the potential antipsychotic-like activity of galantamine and donepezil, respectively. Further incentive for these studies was provided by more recent clinical data reporting that adjunctive galantamine or donepezil, besides effect on cognition also may improve negative and, to some extent, positive symptoms in schizophrenia (Keefe et al., 2007; Norén et al., 2006). Therefore, the effect of the adjunctive treatment of galantamine and donepezil, respectively, to the selective dopamine  $D_{2/3}$  antagonist raclopride was studied in the CAR test. EPS liability was also assessed by means of the catalepsy test.

We observed that galantamine alone could not suppress CAR without inducing escape failures. Galantamine (1.0 mg/kg, s.c.) augmented the raclopride (0.075 mg/kg, s.c.)-induced suppression of CAR without inducing escape failures, however the highest dose of galantamine (5.0 mg/kg, s.c.) significantly enhanced the raclopride-induced suppression of CAR, but at the same time caused a massive increase in escape failures (figure 18).



We also observed that donepezil alone could not suppress CAR without inducing escape failures. Pretreatment with donepezil (5.0 mg/kg, s.c.) significantly enhanced the raclopride (0.075 mg/kg, s.c.)-induced suppression of CAR (figure 19), however this suppression of CAR was associated with a massive increase in escape failures. The combination of 1.0 mg/kg of donepezil and raclopride produced a statistically significant increase in escape failures (figure 19).



To evaluate the potential EPS liability of galantamine and donepezil, respectively, alone and combined with raclopride, we also studied catalepsy in rats. Neither galantamine (0.1, 1 or 5 mg/kg, s.c.) nor raclopride (0.75 mg/kg, s.c.), alone or in combination, induced any catalepsy (table 1). In contrast, we found that the highest dose of donepezil (5.0 mg/kg, s.c.), even when given alone, induced mild but significant catalepsy. In addition, when this dose of donepezil was combined with 0.75 mg/kg of raclopride a moderate and significant catalepsy was observed (table 2).

Table 1. Effects of galantamine, alone or in combination with raclopride, on catalepsy scores.

Treatment (mg/kg)	Observation Time (min)		
	30	60	120
Saline / Saline	0	0	0
Gal 0.1 / Saline	0	0	0
Gal 1 / Saline	0	0	0
Gal 5 / Saline	0 ± 0.25	0 ± 0.5	0
Saline / Rac 0.075	0 ± 0.25	0	0
Gal 0.1 / Rac 0.075	0 ± 0.25	0	0
Gal 1 / Rac 0.075	0	0	0
Gal 5 / Rac 0.075	0.5 ± 1	0.5 ± 0.5	0

Shown are medians ± semi-interquartile ranges based on observations of eight animals per treatment group. Gal = Galantamine, Rac = Raclopride, Sal = Saline.

Table 2. Effects of donepezil, alone or in combination with raclopride, on catalepsy scores.

Treatment (mg/kg)	Observation Time (min)		
	30	60	120
Saline / Saline	0	0	0
Don 0.1 / Saline	0	0	0
Don 1 / Saline	0	0 ± 0.25	0
Don 5 / Saline	0	0	0.5 ± 0.5 #
Saline / Rac 0.075	0 ± 0.25	0	0
Don 0.1 / Rac 0.075	0	0	0
Don 1 / Rac 0.075	0 ± 0.25	0 ± 0.25	0
Don 5 / Rac 0.075	2.5 ± 2.25 *	1.5 ± 1.5 *	1 ± 0.75 *

Shown are medians ± semi-interquartile ranges based on observations of eight animals per treatment group. #p<0.05 compared with saline/saline and \*p<0.05 compared with saline/raclopride respectively. Don = Donepezil, Rac = Raclopride.

This study showed that a low dose of galantamine significantly augmented the raclopride-induced suppression of CAR, without increasing escape failures. In addition, combined treatment with raclopride and galantamine did not affect catalepsy scores. In contrast, donepezil could not augment the raclopride-induced suppression of CAR without producing both escape failures and significant catalepsy. The differences between galantamine and donepezil may be

related to their different mechanisms of action. Whereas donepezil, as previously mentioned, is a potent and selective inhibitor of AChE, galantamine provides only weak inhibition of AChE but acts as a relatively potent allosteric modulator of nAChRs (Schrattenholz et al., 1996; Thomsen and Kewitz, 1990). Thus, our data propose that galantamine by means of allosteric modulation of nAChRs may enhance the antipsychotic activity when added to typical D<sub>2/3</sub> antagonists without increasing the risk of EPS. Several sets of clinical and preclinical data indicate that  $\alpha 7$  nAChRs are implicated in the pathophysiology of schizophrenia. Generally, stimulation of nAChRs, particularly  $\alpha 7$  nAChRs, may contribute to improve various symptoms of schizophrenia. Previously,  $\alpha 7$  nAChRs have previously been shown to play an important role in the mechanism of normal auditory gating (Luntz-Leybman et al., 1992), while impaired auditory gating in schizophrenia, which has been postulated to contribute to cognitive impairment as well as hallucinations, (Adler et al., 1998), has been linked to polymorphisms in the  $\alpha 7$  nAChR gene (Freedman et al., 1997). Moreover, the partial  $\alpha 7$  nicotinic agonist 3-[(2,4-dimethoxy)benzylidene]anabaseine (DMXB-A) has been shown to reduce cognitive deficits in schizophrenia (Olincy et al., 2006). Consequently, allosteric potentiation of nAChRs by galantamine may contribute to alleviate also other than psychotic symptoms in schizophrenia.

## 5 SUMMARY AND CONCLUDING REMARKS

The present set of studies demonstrate that two different antidepressant drugs with different mechanisms of actions, namely reboxetine and mianserin, can augment the antipsychotic-like effect of raclopride without affecting the raclopride-induced catalepsy. These changes in behavior were in both cases associated with a preferentially increased prefrontal dopamine output. We have also shown that the addition of a selective  $\alpha_2$  adrenoceptor antagonist, idazoxan, can enhance the antipsychotic-like effect of both a typical (haloperidol) and an atypical (olanzapine) antipsychotic drug, which both lack appreciable affinity for  $\alpha_2$  adrenoceptors, and again associated with a preferentially increased dopamine output. Moreover, the addition of idazoxan was found to reverse the haloperidol-induced catalepsy. Our data also demonstrate that the cognitive-enhancing drug galantamine increases VTA dopaminergic neuronal activity *in vivo* via allosteric potentiation of nAChRs and, moreover, increases prefrontal dopamine output. Finally, we have shown that galantamine, but not the selective AChE inhibitor donepezil, can augment the antipsychotic-like effect of raclopride without inducing catalepsy. The results support the notion that increasing prefrontal outflow of dopamine and noradrenaline, either by inhibition of the noradrenaline reuptake mechanism or by blocking the noradrenergic autoreceptor, can enhance the clinical effect of typical and atypical antipsychotic drugs. Blockade of the noradrenaline transporter increases the level of extracellular dopamine, since extracellular dopamine in the PFC is largely inactivated by the noradrenaline transporter (Carboni et al., 1990). Blockade of the  $\alpha_2$  autoreceptor results in increased extracellular levels of dopamine released from noradrenergic terminals (Devoto et al., 2001). This increase in dopamine outflow is more pronounced in the medial PFC than in the NAC since noradrenergic terminals as well as  $\alpha_2$  adrenoceptors are abundant in the PFC but virtually absent in the NAC. Generally, several pharmacological properties of mianserin, in particular blockade of the 5-HT<sub>2A/C</sub> receptors as well as  $\alpha_2$  and  $\alpha_1$  adrenoceptors may contribute to augment the effect of typical antipsychotic drugs in the treatment of schizophrenia. This augmentation appears clinically to comprise not only an effect on positive and negative symptoms, but also alleviation of akathisia and potentially also cognitive dysfunction. Additionally, our results indirectly propose that the increased prefrontal dopamine output caused by allosteric potentiation of nAChRs may contribute to generate enhancement of the antipsychotic effect of typical D<sub>2</sub> antagonists, such as raclopride. Moreover, since the antipsychotic-enhancing effect of galantamine is in all probability a result of allosteric potentiation of nAChRs, particularly  $\alpha_7$  nAChRs, and not inhibition of AChE, our results suggest the development of selective allosteric potentiators of  $\alpha_7$  nAChRs for treatment of schizophrenia.

Although two antidepressant drugs, mianserin and reboxetine, both may allow augmentation of antipsychotic drugs in schizophrenia, other antidepressants may not necessarily function as effectively in this regard. Thus, the adjunctive use of selective serotonin reuptake inhibitors in combination with antipsychotic drugs in the treatment of schizophrenia has shown at best mixed results (Kasckow et al., 2001; Sepehry et al., 2007; Silver and Nassar, 1992; Spina et al., 1994; Taiminen et al., 1997). Interestingly, previous experimental data have demonstrated that repeated treatment with selective noradrenaline reuptake inhibitors, but not selective serotonin reuptake inhibitors, generates an increased dopamine output in the PFC (Tanda et al., 1996), suggesting in principle that this mechanism may indeed be causally related to the augmentation obtained with mianserin and reboxetine. The fact that quetiapine has been found clinically effective already at a D<sub>2</sub> receptor occupancy in brain of around 45%, may be interpreted as further support for this notion, since quetiapine generates a metabolite, nor-quetiapine, which is a potent noradrenaline reuptake inhibitor. This interpretation is congruent with the fact that clozapine, which is a potent  $\alpha_2$  adrenoceptor antagonist, dramatically increases prefrontal dopamine outflow, shows clinical efficacy at similar, low levels of D<sub>2</sub> receptor occupancy (see Hertel et al., 1999a). Further support for this notion is provided by the clinical observation that low, but not high, doses of L-DOPA when given as adjunctive treatment to typical antipsychotic drugs may augment their effect in schizophrenia, particularly on negative and cognitive symptoms (Jaskiw and Popli, 2004), since experimental data show that also this drug combination generates a preferential increase in prefrontal dopamine outflow (Eltayb et al., 2005).

The above mentioned conclusions appear to fit several sets of data which indicate an impaired prefrontal dopamine functioning in schizophrenia. Thus, for example, dysfunctional D<sub>1</sub> receptors in the PFC have been suggested to be involved in the pathophysiology of working memory in schizophrenia (Davis et al., 1991; Goldman-Rakic, 1994; Goldman-Rakic et al., 2000; Weinberger, 1987) and substantial evidence indicates that cognitive processes are indeed dependent on normally functioning D<sub>1</sub> receptors in the frontal cortex (Goldman-Rakic et al., 2000). Interestingly, D<sub>1</sub> receptors have been reported to be down-regulated in the PFC in both drug naïve and medicated schizophrenic patients (Okubo et al., 1997a; Okubo et al., 1997b; Sedvall and Farde, 1995). This may partly be explained by the finding that D<sub>1</sub> receptors become down-regulated in the PFC in non-human primates after long-term D<sub>2</sub> blockade by for example antipsychotic drugs (Lidow et al., 1997; Lidow and Goldman-Rakic, 1994; Lidow et al., 1998). Interestingly, clozapine has been shown to act as a partial D<sub>1</sub> agonist (Salmi and Ahlenius, 1996; Salmi et al., 1994), and the antipsychotic-induced cognitive impairment due to down-regulated D<sub>1</sub> receptors could be reversed by D<sub>1</sub> receptor stimulation (Castner et al., 2000).

Increasing the levels of prefrontal dopamine by atypical antipsychotic drugs or by augmentation strategies as demonstrated in this thesis may thus improve cognitive function by stimulation of prefrontal D<sub>1</sub> receptors, which in turn secondarily facilitates glutamatergic neurotransmission in the PFC as we have previously shown (Marcus et al., 2005). Against this background, it seems likely that also the increased dopamine output in the PFC caused by galantamine may contribute to the augmentation of antipsychotic drugs seen in our experimental paradigm. This effect is in all probability related to allosteric potentiation of nAChRs, a mechanism that also has bearing on other symptoms and functional deficits in schizophrenia, such as reduced capacity for sensory gating and cognitive impairment. Consequently, selective allosteric potentiators of  $\alpha 7$  nAChRs may provide an important new addition to the armamentarium of drugs that can be used in schizophrenia. In this regard our data suggest that such drugs, if used as adjunctive treatment together with antipsychotic drugs, may actually enhance the antipsychotic effect *per se* and tentatively allow for dose reduction and less D<sub>2</sub> occupancy in brain with maintained efficacy.

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