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Utility of Combined Treatment with Antipsychotic and Antidepressant Drugs: Scientific Rationales and Clinical Implications

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ABSTRACT

The atypical antipsychotic drug (APD) clozapine is the most efficacious APD in treatment-resistant schizophrenia including negative symptoms and cognitive impairment, and still lacks extrapyramidal side effects (EPS). Clozapine, which also possesses an antidepressant effect and can be used as monotherapy in bipolar disorder, has a broad receptor binding profile with higher affinity for the α_2-adrenoceptor and several serotonergic receptors than D_2 receptor, enhances dopamine output in the medial prefrontal cortex (mPFC) and facilitates glutamatergic NMDA receptor-mediated transmission in pyramidal cells in the same brain region. These effects may clearly contribute to its superior clinical efficacy, although haematological side effects limit its use. The atypical APD olanzapine lacks e.g. the high affinity to the α_2-adrenoceptor, as well as the high efficacy of clozapine, and generates dose dependent EPS. Previous studies show that addition of the selective α_2-adrenoceptor antagonist idazoxan to olanzapine may enhance its antipsychotic-like effect and increase dopamine output in the mPFC, effects that might also be achieved by inhibition of the norepinephrine transporter (NET). In the present study we investigated whether adjunct treatment with reboxetine, a selective NET inhibitor used for the treatment of depression, might generate another means to augment the antipsychotic-like effect of olanzapine and, in principle, provide a somewhat more clozapine-like effect. Addition of reboxetine potentiated the antipsychotic-like effect of low doses of olanzapine, without increasing EPS liability. This combined treatment also preferentially enhanced cortical dopamine output and NMDA receptor-mediated currents in pyramidal cells of the mPFC in slice preparations. The results propose that adjunct NET inhibition by reboxetine may be used to augment the antipsychotic effect of low doses of olanzapine in schizophrenia and improve the effect on negative symptoms and cognitive impairments. We continued to experimentally investigate whether NET inhibition by norquetiapine, an active metabolite of quetiapine in humans but not in rodents, and a potent NET inhibitor, may contribute to the overall effects of quetiapine in patients. To this end we studied the effects of reboxetine added to quetiapine in rodents and found an augmented antipsychotic-like effect and a selectively enhanced dopamine output in the mPFC. As the increased extracellular dopamine levels in the mPFC were accompanied by a decrease in DOPAC levels, the enhanced extracellular dopamine levels should represent a consequence of NET inhibition. Although high concentrations of quetiapine alone facilitated NMDA-induced currents in the mPFC, concomitant NET inhibition was found to generate the same effect at a low, subeffective concentration of quetiapine, being mediated via the dopamine D_1 receptor. Consequently, NET inhibition generated by the active metabolite norquetiapine in patients should, in principle, contribute to the clinical antipsychotic effect of quetiapine, which is obtained at low D_2 receptor occupancy, and furthermore serve to improve depressive symptoms as well as cognitive impairments. Low to moderate doses of atypical APDs added to selective serotonin reuptake inhibitors (SSRIs) have been found to augment the antidepressant effect with a rapid onset compared to SSRIs alone. Our data show that addition of low doses of the novel atypical APD asenapine to the SSRI escitalopram enhances the output of monoamines in the mPFC and also facilitates not only NMDA, but also AMPA receptor-mediated transmission in pyramidal cells of the mPFC, both effects being mediated via activation of the dopamine D_1 receptor. A similar effect was also obtained by a combination of low concentrations of olanzapine and the SSRI fluoxetine. Significantly, a systemic ketamine injection 24 hours prior to the electrophysiological experiments, which previously has been found to produce a rapid and potent antidepressant-like effect in rodents, significantly potentiated AMPA receptor-mediated transmission in the mPFC in our study. Consequently, our data propose that asenapine may be clinically used as adjunct to SSRIs in treatment-resistant depression to augment and hasten the clinical response. Overall our data thus propose that the relatively rapid onset of the augmented antidepressant effect of combined antipsychotic and antidepressant drug treatments may be related to an enhanced AMPA receptor-mediated transmission in the PFC, in analogy with the effects of ketamine. In summary, our experimental results suggest that an enhanced efficacy in both schizophrenia and depression may be achieved by combined administration of atypical APDs and antidepressant drugs.

LIST OF PUBLICATIONS

I. Marcus MM, Jardemark K, Malmerfelt A, **BJÖRKHOLM C** and Svensson TH (2010). Reboxetine Enhances the Olanzapine-Induced Antipsychotic-Like Effect, Cortical Dopamine Outflow and NMDA Receptor-Mediated Transmission. *Neuropsychopharmacology*. 35:1952-1961.

II. **BJÖRKHOLM C**, Jardemark K, Marcus MM, Malmerfelt A, Nyberg S, Schilström B, Svensson TH (2013). Role of concomitant inhibition of the norepinephrine transporter for the antipsychotic effect of quetiapine. *Eur Neuropsychopharmacol.* 23:709-720.

III. **BJÖRKHOLM C**, Frånberg O, Malmerfelt A, Marcus MM, Konradsson-Geuken Å, Schilström B, Jardemark K, Svensson TH. Adjunctive treatment with asenapine augments the escitalopram-induced effects on monoaminergic outflow and glutamatergic neurotransmission in the medial prefrontal cortex. *Manuscript.*

IV. **BJÖRKHOLM C**, Schilström B, Jardemark K, Svensson TH. Effects of a combination of olanzapine and fluoxetine as well as ketamine on AMPA and NMDA receptor-mediated transmission in the medial prefrontal cortex of the rat. *Manuscript.*

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LIST OF ABBREVIATIONS

3-MT	3-metoxytyramine
5-HIAA	5-hydroxyindole acetic acid
5-HT	5-hydroxytryptamine (serotonin)
AD	Aldehyde dehydrogenase
AMPA	2-amino-3-(3-hydroxy-5-methyl-isoxazol-4-yl) propionic acid
ANOVA	Analysis of variance
APD	Antipsychotic drug
AUC	Area under the curve
CAR	Conditioned avoidance response
CaMKII	Calcium/calmoduline-dependent kinase II
c.f.	Compare (*Confer* lat)
CNS	Central nervous system
CNQX	6-cyano-7-nitroquinoxaline-2,3 dione
COMT	Catecholamine-O-metyltransferase
CS	Conditioned stimulus
CSF	Cerebrospinal fluid
DAG	Diacylglycerol
DARPP-32	Dopamine and cAMP-regulated phosphoprotein of 32-kDa
DAT	Dopamine transporter
dlPFC	Dorsolateral prefrontal cortex
DOPAC	Dihydroxyphenylacetic acid
DRN	Dorsal raphe nucleus
e.g.	For example (*exempli grata* lat)
EPS	Extrapyramidal side effects
EPSC	Excitatory postsynaptic current
EPSP	Excitatory postsynaptic potential
GABA	γ-aminobutyric acid
HPLC	High performance liquid chromatography
HVA	Homovanillic acid
i.e.	That is (*Id est* lat)
i.p.	Intraperitoneally
IP_3	Inositol triphosphate
i.v.	Intravenously
LC	Locus coeruleus
L-DOPA	L-dihydroxyphenylalanine
LSD	Lysergic acid diethylamide
MAO	Monoamine oxidase
MDD	Major depressive disorder
MHPG	3-methoxy-4-hydro-phenylglycol
MK-801	Dizoclipine
mPFC	Medial prefrontal cortex
mTOR	Mammalian target of rapamycin

NAc	Nucleus accumbens
NET	Noradrenaline transporter
NMDA	N-metyl-D-aspartate
PCP	Phencyclidine
PET	Positron emission tomography
PFC	Prefrontal cortex
PKA	Protein kinase A
PKC	Protein kinase C
s.c.	Subcutaneously
S.E.M	Standard error of the mean
SN	Substantia nigra
STR	Striatum
TCA	Tricyclic antidepressant
TTX	Tetrodotoxin
UCS	Unconditioned stimulus
VMAT	Vesicular monoamine transporter
VTA	Ventral tegmental area

1. Introduction

Mental illness has been defined as "health conditions that are characterized by alterations in thinking, mood, or behavior (or some combination thereof) associated with distress and/or impaired functioning" (HHS, 1999). In a study investigating the global burden of disease, psychiatric disorders occupied five places on the top-ten list of the leading causes of disability in the world, when calculated as years lived with a disability (Lopez and Murray, 1998). In addition to immense personal suffering for the afflicted and his next of kin, mental illness poses an enormous cost to society with regards to direct and indirect medical cost (Gustavsson et al., 2011, Wittchen et al., 2011).

The discovery of several new drugs (e.g. chlorpromazine, lithium and imipramine) revolutionized psychiatric care during the latter half of the 20th century and changed the life for large patient groups that previously had been without rational treatments and been confined to hospitalization. Even though many drugs have been developed since then, the efficacy and side effect profile of the drugs available today are by no means optimal.

Approximately one third of patients suffering from depression achieve remission with selective serotonin reuptake-inhibitors, the most prescribed antidepressant drugs (Trivedi et al., 2006). Moreover, a recent Swedish study showed that, although there are a number of effective antipsychotic drugs (APDs) available, schizophrenia is still so debilitating that less than one in fourteen schizophrenic patients are employed, suicide rates are increased ten-fold and schizophrenic patients are estimated to die 12 to 15 years earlier than the rest of the population as a consequence of their disease (Crump et al., 2013). Thus, improved treatments for these diseases are urgently needed. In the present thesis, using preclinical methodologies, I have investigated augmentation strategies to improve the treatment of schizophrenia and mood disorders by combining conventional antidepressant drugs and APDs.

1.1. Neurotransmitter systems

1.1.1. The dopamine system

In 1957, Montagu claimed to have identified dopamine in brain tissue from several species (Montagu, 1957). However, it was Carlsson and co-workers who originally discovered that dopamine has a physiological function as a neurotransmitter in its own right, not just serving as a precursor in the synthesis of noradrenaline (Carlsson et al., 1957, Carlsson et al., 1958, Carlsson, 1959). Since its discovery, dopamine has been found to be critically involved with a multitude of complex behaviors such as reward, cognition, salience and movement control. Aberrant dopaminergic transmission is thought to be involved in several central nervous system (CNS) disorders, accordingly, drugs affecting dopaminergic transmission are used in e.g. schizophrenia, Parkinson's disease and mood disorders.

1.1.2. The dopamine pathways

In the CNS, the dopaminergic system can be divided into four distinct pathways; namely the nigrostriatal, the mesocortical, the mesolimbic and the tuberoinfundibular pathway (Dalström and Fuxe, 1964, Ungerstedt, 1971, Moore and Bloom, 1978; figures 1, 2). In the nigrostriatal pathway, the cell bodies are located in the substantia nigra (SN; A9), and project predominantly to the dorsal striatum (STR), i.e. caudate and putamen, where they form extensive axonal arborizations which can measure up to 500 mm in length (Matsuda et al., 2009). The nigrostiatal pathway is involved in movement control, and Parkinson's disease is largely caused by cell death in this pathway (Hornykiewicz, 1962). Moreover, massive blockade of D_2 receptors in the dorsal striatum may induce extrapyramidal side effects, (EPS) such as Parkinsonism, which is a common side effect of most, but not all APDs, especially in higher doses (see 3.4).

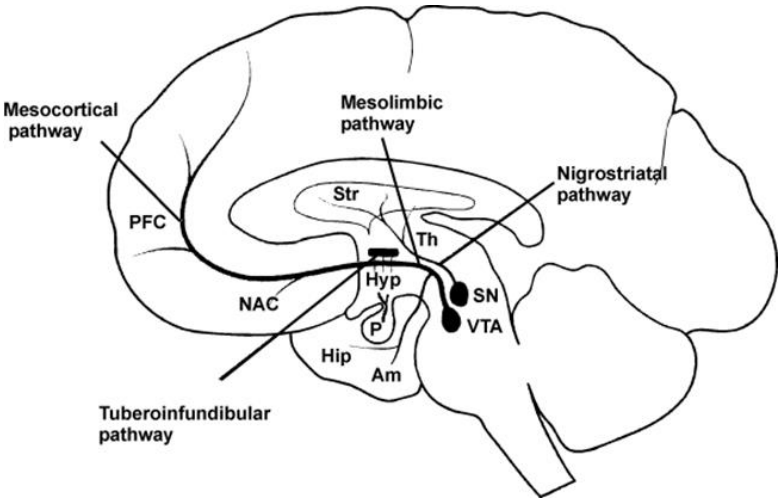

Figure 1. *Dopaminergic pathways in the human brain. AM: amygdala; Hip: hippocampus; Hyp: hypothalamus; NAC: Nucleus accumbens; P: pituitary gland; PFC: prefrontal cortex; SN: substantia nigra; Th: Thalamus; VTA: ventral tegmental area. Modified from (Rang et al., 1999).*

Dopamine cells that originate in the ventral tegmental area (VTA; A10) constitute the other main dopaminergic pathway, the mesocorticolimbic pathway. Depending on where the dopamine neurons in the VTA project, the mesocorticolimbic pathway can be further divided into the mesocortical part (projecting to the prefrontal cortex [PFC]) and the mesolimbic part projecting to subcortical brain regions e.g. the ventral STR (i.e. the nucleus accumbens [NAc]), amygdala and the hippocampus. The pituitary gland and the median eminence receive dopaminergic input from the arcuate nucleus in the hypothalamus and this pathway constitute the tuberoinfundibular system which acts inhibitory on the prolactin synthesis and secretion, via activation of D_2 receptors (Fitzgerald and Dinan, 2008). Thus, blockade of D_2 receptors, by APDs (especially typical APDs) may increase the secretion of prolactin, causing side effects such as galactorrhea.

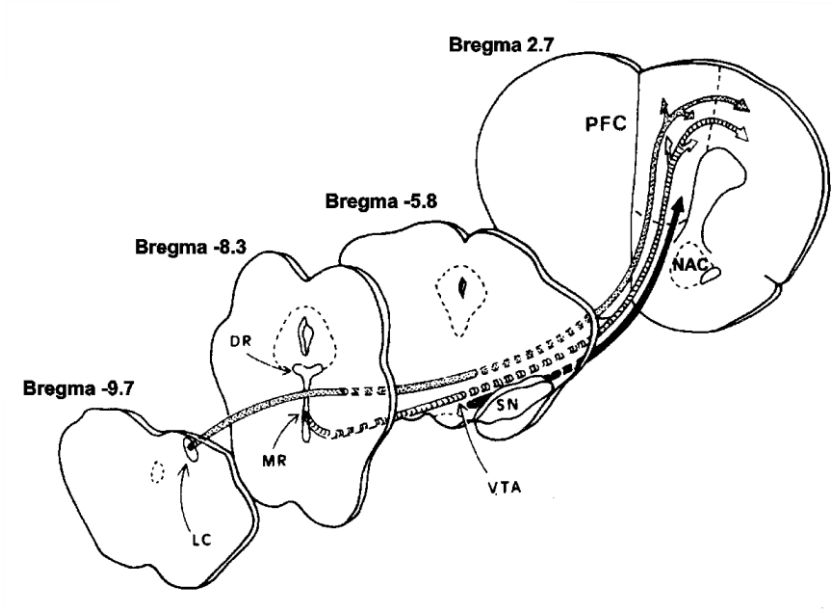

Figure 2. *Schematic drawing showing monoaminergic pathways innervating the prefrontal cortex (PFC) on coronal sections of rat brain. The noradrenergic pathway projects from the locus coeruleus (LC), the serotonergic pathway from the dorsal and median raphe (DR, MR) and the dopaminergic pathway from ventral tegmental area (VTA). NAC: Nucleus accumbens; SN: Substantia nigra. Modified from (Fuster, 1997).*

1.1.3. Dopamine synthesis and elimination

Dopamine is synthesized from the amino acid tyrosine, which is actively transported over the blood brain barrier into the brain (Brunton et al., 2011). Tyrosine is first converted to L-dihydroxyphenylalanine (L-DOPA) by the enzyme tyrosine hydroxylase before L-DOPA is converted into dopamine by the enzyme dopa-decarboxylase (figure 3). Dopamine is transported into synaptic vesicles by the vesicular monoamine transporter2 (VMAT2), which is located in the vesicular membrane. The drug reserpine inhibits VMAT2 and thereby inhibits the transport of monoamines into vesicles, which depletes the terminals of monoamines. Dopamine is released into the synaptic cleft when vesicles are fused with the cell membrane (exocytosis) via a Ca^{2+}-dependent mechanism initiated by nerve impulses. Once released into the synaptic cleft, the main elimination route of dopamine is reuptake back into the dopaminergic terminal by the dopamine transporter (DAT). Blockade of the DAT causes a large increase in extracellular dopamine levels and represents a major mechanism of action of several drugs such as cocaine and bupropion. When dopamine is transported back into the terminal, it can either be packed into vesicles and reused or metabolized to dihydroxyphenyl acetic acid (DOPAC) by the enzymes monoamine oxidase (MAO) and aldehyde dehydrogenase. In the extracellular space, dopamine can also be metabolized by catechol-O-methyl transferase (COMT) into 3-metoxytyramine (3-MT). 3-MT and DOPAC may also be further metabolized into homovanillic acid (HVA), by MAO and COMT respectively. There are two isoforms of the MAO enzyme, MAO-A and MAO-B. MAO-A has higher affinity for serotonin, whereas both dopamine and noradrenaline show equal affinity for these enzymes (Waldmeier, 1987, Berry et al., 1994). MAO-A inhibitors are used as antidepressant drugs, whereas MAO-B inhibitors are preferentially used in the treatment of Parkinson's disease.

1.1.4. Dopamine receptors

The dopamine receptors are divided into two families depending on their structural, pharmacological and signaling properties (see Beaulieu and Gainetdinov, 2011, Tritsch and Sabatini, 2012, and references therein). The D_1– like family consists of D_1 and D_5 receptors and the D_2- like family consists of D_2, D_3 and D_4 receptors. Pharmacological agents can distinguish between the two families, but usually possess less specificity within each family. In general, stimulation of D_1-like and D_2-like receptors exert opposite effects by activation of different second messenger pathways. Dopamine has been found to possess a higher affinity for the D_2-like receptors than the D_1-like receptors.

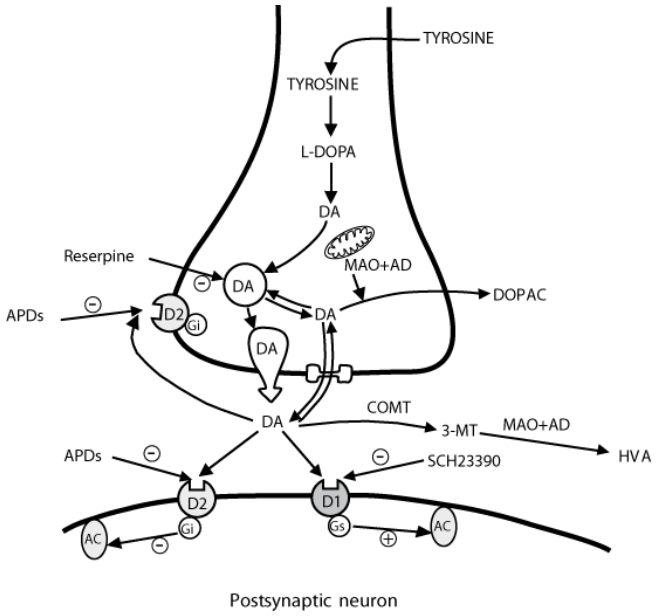

Figure 3. *Schematic drawing illustrating a dopaminergic nerve terminal. 3-MT:* 3-metoxytyramine; *AD: aldehyde dehydrogenase; APDs: antipsychotic drugs; COMT: catecholamine-O-methyl transferase; DA: dopamine; MAO: monoamine oxidase. Modified from (Cooper et al., 2003).*

Binding of dopamine to the D_1 receptor activates $G_{\alpha s}$ or $G_{\alpha/olf}$, which are positively coupled to adenylyl cyclase and thereby increases cyclic adenosine monophosphate (cAMP) production (Brunton et al., 2011). Increased levels of cAMP may subsequently activate protein kinase A (PKA). D_1 receptor activation is also suggested to couple to G_q and enhance the production of inositol triphosphate (IP_3) and diacylglycerol (DAG). PKA mediates most of the effects of D_1 receptor stimulation, and PKA may in turn regulate the function of several different cellular substrates, such as voltage gated ion channels, ionotropic glutamate receptors, γ-aminobutyric acid (GABA)-ergic receptors and transcription factors. PKA may also activate the dopamine and cAMP-regulated phosphoprotein of 32-kDa (DARPP-32). This effect on DARPP-32 can be inhibited by activation of D_2 receptors. D_2 receptor activation inhibits adenylyl cyclase activation but may also affect intracellular Ca^{2+} levels and voltage-gated ion channels independently of cAMP/PKA inhibition. D_2 receptors are expressed presynaptically as well as postsynaptically. D_2 receptors located on the soma and dendrites of dopaminergic cells act as autoreceptors, decreasing firing frequency (see below) whereas D_2 receptors located on nerve terminals reduce dopamine synthesis and release. D_2 receptors exist in two splice variants D_{2S} (short) and D_{2L} (long). D_{2L} is predominantly located

postsynaptically whereas D_{2S} is predominantly expressed presynaptically (Brunton et al., 2011).

Dopamine receptors are expressed in regions that receive dopaminergic innervations. The most commonly expressed receptor subtypes are the D_1 and the D_2 receptors, with the D_1 being the most widely distributed and abundant. In subcortical areas, the expression of D_1 and D_2 are approximately equal whereas in the cortex, the D_1 type outnumbers the D_2. Dopamine receptors (D_1 and D_2 subtypes) are expressed on medium spiny neurons as well as interneurons in the STR and on as on cortical pyramidal cells, interneurons and glial cells (Tritsch and Sabatini, 2012). The D_3 receptor shows a lower expression than the D_2 receptor and is predominantly found in limbic regions. The D_4 and D_5 receptors are expressed in e.g. cortical regions but also limbic areas (Tritsch and Sabatini, 2012).

1.1.5. Regulation of dopamine cell activity

Midbrain dopamine cells (i.e. located in SN or VTA) essentially display two modes of function, single spike firing and burst firing i.e. short bursts of action potentials with high frequency (Bunney et al., 1973, Grace and Bunney, 1983). Burst firing is associated with a larger release of dopamine in both cortical and subcortical areas (Gonon, 1988, Bean and Roth, 1991, Chergui et al., 1996) and may be especially important for signaling reward or salience (Schultz, 2010). Single spike firing on the other hand, may provide a basal tonic stimulation of dopaminergic receptors which is important for e.g. motor activity (Schultz, 2007).

Activation of somatodendritic D_2 receptors on midbrain dopamine cells hyperpolarizes the neurons and reduces their firing rate by enhancing K^+ conductance (Bunney et al., 1973, Lacey et al., 1987). However, the mesocortical dopamine cells are not regulated by autoreceptors (Chiodo et al., 1984). The cortically projecting dopamine cells also respond differently to e.g. N-methyl-D-aspartate (NMDA) receptor antagonists than the mesolimbic dopamine neurons (see e.g. Murase et al., 1993b), have higher firing frequencies and fire a larger proportion of spikes in bursts compared with dopamine cells in the mesolimbic or nigrostriatal pathways. In addition, mesolimbic dopamine release appears to be subjected to negative feed-back control by dopamine in the mPFC (Pycock et al., 1980, Deutch et al., 1990). Thus, mesocortical and mesolimbic dopamine cells are differentially regulated in several ways. Moreover, the midbrain dopamine cells are negatively modulated by GABAergic interneurons as well as GABAergic feedback loops originating in brain regions innervated by dopamine such as the STR and the NAc (Fonnum et al., 1978, Walaas and Fonnum, 1980).

Dopamine cells receive excitatory input from the PFC but also from e.g. the subthalamic nucleus (Grace and Bunney, 1985, Svensson and Tung, 1989, Chergui et al., 1994). This is indicated by experiments showing that inactivation of the mPFC reduces burst firing, whereas activation of the mPFC increases the proportion of spikes fired in bursts in dopamine cells in the VTA (Gariano and Groves, 1988, Svensson and Tung, 1989, Murase et al., 1993a). The dopamine cells in the VTA also receive a

noradrenergic input from locus coeruleus (LC), neurons which may enhance burst activity via activation of excitatory α_1-adrenoceptors on the dopaminergic cell bodies (Grenhoff et al., 1993, Grenhoff and Svensson, 1993). The VTA receives serotonergic afferents from the raphe nuclei and although the modulation of dopamine firing in the VTA is complex, the main effect seems to be inhibitory (Di Giovanni et al., 2008).

1.1.6. Regulation of dopamine in the cortex

The expression of DAT is scarce in the PFC, in contrast to the abundant DAT expression in other dopaminergic terminal areas, such as the STR (Sesack et al., 1998). As a consequence, prefrontal dopamine is essentially inactivated by the norepinephrine transporter (NET) located in noradrenergic nerve terminals (Carboni et al., 1990, Pozzi et al., 1994). As a result, NET-inhibitors increase both dopamine and noradrenaline levels in the mPFC to a similar extent, but does not affect dopamine levels in NAc or STR where dopamine is cleared by the DAT (Bymaster et al., 2002). Furthermore, blockade of the α_2-adrenoceptor in the mPFC increases the extracellular levels of dopamine (Hertel et al., 1999b). In fact, lesion and pharmacological studies indicate that dopamine may be co-released with noradrenaline from noradrenaline terminals in the mPFC (Devoto et al., 2001, Devoto and Flore, 2006, Masana et al., 2011).

1.2. The glutamate system

Glutamate is the main excitatory neurotransmitter in the CNS and is found in high concentrations throughout the brain. It is estimated that approximately 80 % of all neurons and 85% of all synapses in the human neocortex are glutamatergic (Douglas and Martin, 2007). Given the almost ubiquitous nature of glutamate, it is involved in almost all processes in the brain, in one way or the other. The majority of the glutamatergic projections is descending and project from the cortex to subcortical regions, but may also project within the cortex (i.e. cortico-cortical projections). In the nerve terminals, glutamate can be synthesized from glucose, via the Krebs cycle, or from glutamine, which is synthesized in glia, and converted to glutamate by glutaminase. Inactivation of released glutamate is accomplished by reuptake into neurons or glia. In glia, glutamate is metabolized into glutamine by glutamine synthease. Glutamine is subsequently transported to neighboring neurons where it is converted to glutamate and subsequently reused. Glutamate receptors include ionotropic receptors, i.e. NMDA, AMPA and kainate receptors, as well as metabotropic glutamate receptors (mGluR 1-7). Glutamate receptors of all types have been found to be located both pre- and postsynaptically (Pinheiro and Mulle, 2008).

1.2.1. NMDA-receptors

The NMDA receptor is a ligand-gated voltage-dependent ionotropic receptor that is widely expressed in the CNS (figure 4). NMDA receptors have slow activation/deactivation kinetics and are highly permeable to Ca^{2+} as well as Na^+ and K^+ (see Cull-Candy et al., 2001 and references therein). NMDA receptors are essential for neuronal development, learning and neural plasticity as well as neural cell death.

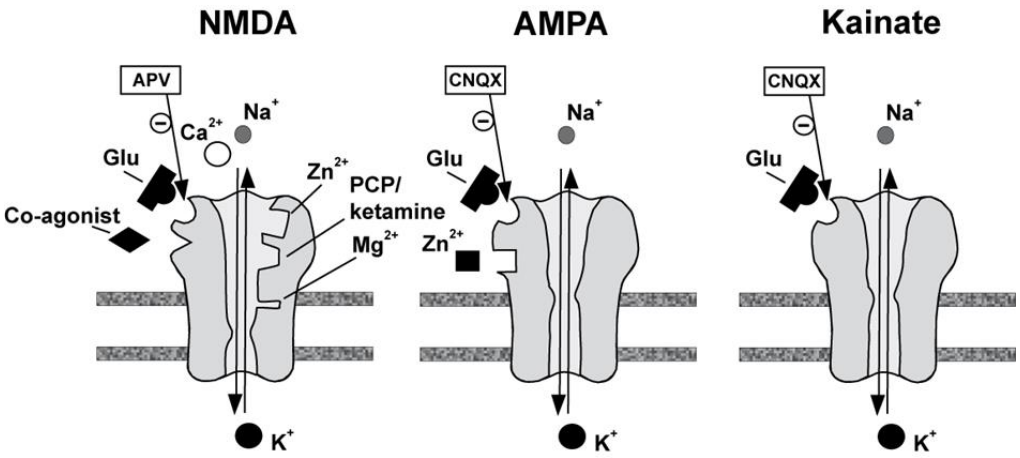

Figure 4. *Schematic drawing of glutamatergic ionotropic receptors. Co-agonist i.e. glycine or D-serine; Glu: glutamate; APV: D(−)-2-Amino-5-phosphonopentanoic acid, a competitive NMDA receptor antagonist; CNQX: 6-Cyano-7-nitroquinoxaline-2,3-dione, a AMPA/ Kainate receptor antagonist. Modified from (Kandel et al., 1991).*

The activity of the NMDA receptor is regulated by several different mechanisms. In addition to glutamate, NMDA receptor activation also requires binding of a co-agonist (glycine or D-serine) controlling the number of NMDA receptors that can be activated by released glutamate (Johnson and Ascher, 1987, Mothet et al., 2000, Oliet and Mothet, 2009). Glycine levels are regulated by glycine transporters which are located on glial cells and glutamatergic neurons close to NMDA receptor synapses (Cubelos et al., 2005, Eulenburg et al., 2005). In addition, NMDA receptor ion-channels are blocked by Mg^{2+} ions at resting membrane potentials and in order for the NMDA receptor to be activated, the membrane potential must be depolarized (Cull-Candy et al., 2001). In the postsynaptic density, NMDA receptors may associate with scaffolding, anchoring and signaling proteins (Cull-Candy et al., 2001).

There is considerable heterogeneity among the NMDA receptors, depending on their subunit composition (Cull-Candy et al., 2001). There are eight different splice variants of the NR1 subunit, four different NR2 subunits and two NR3 subunits. The NMDA receptor is considered to be a tetramer, the most common consisting of two NR1 subunits and two NR2 subunits, which can be of different splice variants. However, the NMDA receptor may also contain NR3 subunits. The subunit composition is important as it determines the pharmacological and biophysical properties of the NMDA receptor. NR2 subunits bind glutamate and contain the modulatory site, binding Zn^{2+}, whereas the NR1 and NR3 subunits contain the co-agonist site. Phencyclidine (PCP), ketamine and MK-801 bind to the pore of the ion-channel and thereby block the transmission.

1.2.2. AMPA and kainate receptors

The AMPA and kainate receptors are ionotropic receptors, which are responsible for the major part of the fast excitatory transmission in the CNS. The AMPA receptors are co-localized with NMDA receptors. Activation of AMPA receptors induces an influx of Na^{+} increasing the membrane potential, which is required to release the Mg^{2+} blockade of the NMDA receptor (see above). Activation of NMDA receptors may regulate the

number of AMPA receptors in the synapse trough Ca^{2+} influx and triggering of intracellular cascades, thus regulating the synaptic strength (i.e. long term depression or long term potentiation; Malinow and Malenka, 2002, Citri and Malenka, 2008). Regulation of synaptic strength is thought to be involved in learning and memory.

The AMPA receptors are composed of four subunits (GluR1 to 4), which each contains a glutamate binding-site (Rosenmund et al., 1998). AMPA receptors may be heteromers as well as homomers (Wenthold et al., 1996). Most AMPA receptors are Na^+ and K^+ permeable to but may also be permeable to Ca^{2+} if the receptor lacks the GluR2 subunit. The kainate receptors form homo- and heterotetramers from the subunits GluR5-7 and KA1 and 2. The kainate receptors are distributed throughout the brain but are less abundant than the AMPA receptors (Pinheiro and Mulle, 2006).

1.2.3. Metabotropic glutamate receptors

There are eight types of metabotropic glutamate receptors (mGluRs) divided into three groups (see Nicoletti et al., 2011, and references therein). Group I includes GluR1 and 5, group II includes mGluR 2 and 3, and subsequently, group III includes mGluRs 4, 6, 7 and 8. mGluRs are expressed on neurons as well as on microglia and astrocytes and are widely expressed throughout the brain. The mGluRs are involved in pre- and postsynaptic regulation of synaptic transmission and are considered as interesting drug targets for the treatment of a number of neuropsychiatric disorders, such as depression, anxiety and schizophrenia. For example, mGluR2/3 agonists, which attenuate glutamate release, have been developed for schizophrenia and initially showed encouraging results (Patil et al., 2007). However, a subsequent trial could not confirm the initial finding (Kinon et al., 2011) and thus the effectiveness of mGlu2/3 as a target for schizophrenia remains to be conclusively determined.

1.2.4. Dopamine D1 receptor and NMDA receptor interactions in the PFC

Several lines of evidence support the functional as well as physical interaction between the dopamine D_1 and the NMDA receptor. In fact, optimal interaction between the D_1 receptor and the NMDA receptor in the PFC has been proposed as a crucial mechanism for cognitive function (Castner and Williams, 2007).

Dopamine projections to the mPFC terminate mainly in deep cortical layers (layer V and VI) and pyramidal cells in layer V receive both dopaminergic and glutamatergic input from the VTA and from the thalamus, respectively (Kuroda et al., 1996). D_1 receptors and NMDA receptors co-localize on pyramidal cells, as well as on interneurons in the rat mPFC (Kruse et al., 2009). Dopamine D_1 receptor activation has been found to facilitate NMDA-induced responses and to potentiate excitatory postsynaptic potentials (EPSPs) in layer V pyramidal cells of the rat mPFC (Seamans et al., 2001, Tseng and O'Donnell, 2004), whereas α- or β-adrenoceptors do not seem to affect NMDA-induced currents (Wirkner et al., 2004). In contrast to the well established interaction between the D_1 and NMDA receptors, interactions between D_1 and AMPA receptors remain to be clarified.

1.3. The serotonin system

Evolutionary, serotonin is thought to be one of the oldest neurotransmitters and is found to in the CNS as well as in the peripheral nervous system and in various non-neural tissues. The distribution of serotonin is widespread in the brain (figure 2 and 5) and serotonin modulates a number of important functions including sleep, mood, aggression, cognition, temperature and feeding. Accordingly, the cerebral serotonin system is a target for the treatment of several psychiatric disorders, such as depression and anxiety.

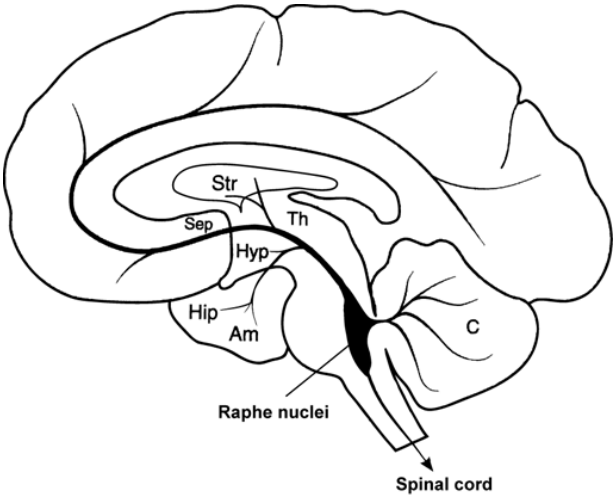

Figure 5. *Schematic drawing illustrating the serotonergic pathways in the human brain. AM: amygdala; C: cerebellum; Hip: hippocampus; Hyp: hypothalamus; Str: striatum; Sep: Septum; Th: Thamalus. Modified from (Rang et al., 1999).*

1.3.1. Serotonins synthesis and elimination

Serotonin is synthesized in serotonergic neurons from tryptophan which is converted into 5-hydroxytryptamine (5-HT; i.e. serotonin), via 5-hydroxytrypophan by the enzymes tryptophan hydroxylase and amino acid decarboxylase, respectively (Figure 6.) (Brunton et al., 2011). In the nerve terminal, serotonin is packed into vesicles and released into the synaptic cleft by exocytosis through a nerve impulse initiated Ca^{2+}-dependent mechanism. The main route of elimination is reuptake by the serotonin transporter (SERT). Serotonin is metabolized by MAO and aldehyde dehydrogenase into its main metabolite 5-hydroxyindole acetic acid (5-HIAA).

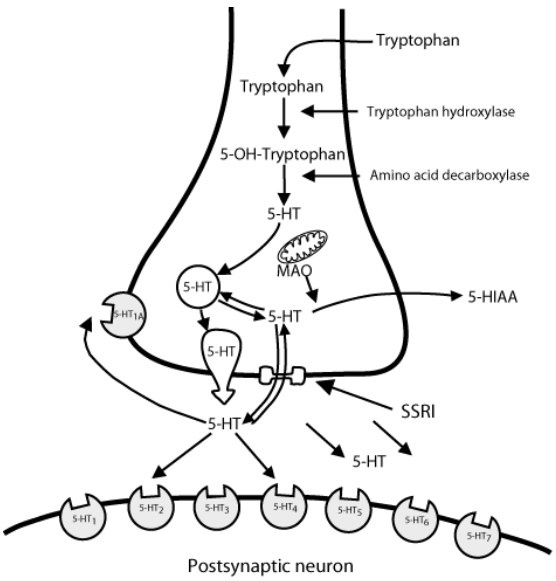

Figure 6. *Schematic drawing illustrating a serotonergic nerve terminal. 5-HT: 5-hydroxytryptamine i.e. serotonin; MAO: monoamine oxidase. Modified from (Cooper et al., 2003).*

1.3.2. Serotonin projections

The serotonergic pathways in the CNS project from the raphe nuclei located in the brain stem (Dahlström and Fuxe, 1964), to most regions of the brain. From the medial and dorsal raphe, serotonergic cells project rostrally to e.g. the thalamus, hypothalamus, striatum, amygdala, hippocampus and the cortex via the medial forebrain bundle (figure 2 and 6) whereas from caudal parts of the raphe nuclei, serotonergic cells project to the cerebellum and the spinal cord.

1.3.3. Serotonin receptors

There are 14 types of serotonergic receptors, 5-HT_{1-7} (with subgroups), all of which are G-protein coupled except for the 5-HT_3 receptor, which is an excitatory ligand-gated ion channel (Hannon and Hoyer, 2008). There are several different subgroups of the serotonin receptors for example 5-$HT1_{A, B, D, E, F}$ and 5-$HT_{2A/B/C}$. The 5-HT_{1A} and 5-$HT_{2A/C}$ receptors are involved in the mechanism of action of many APDs see e.g. (Ichikawa et al., 2001). 5-HT_{1A} receptors are mostly linked to G_i and may hyperpolarize the cell membrane and reduce adenylate cylase. 5-HT_{1A} receptors are expressed in e.g. the hippocampus and in cortical areas as well as on cell bodies in the raphe nuclei, where it acts as an autoreceptor. 5-HT_2 receptors are preferentially G_q coupled and increases IP_3 and PKC, which subsequently enhances the intracellular Ca^{2+} concentration. 5-HT_{2A} receptors are expressed on e.g. cortical pyramidal cells and interneurons as well as in the brain stem, limbic areas and in the basal ganglia. 5-HT_{2B} receptors are expressed in lower number than the 5-HT_{2A} and 5-HT_{2C}, and confined to discrete regions e.g. the medial amygdala where 5-HT_{2B} activation induce anxiolytic effects in rodents. 5-HT_{2C} receptors are expressed in limbic structures and in substantia nigra as well as in some cortical structures.

1.4. The noradrenaline system

Noradrenaline was first identified as a CNS neurotransmitter in the 1950's (Vogt, 1954). Noradrenaline has been found to modulate the activity of neurons, more specifically noradrenaline may function to enhance signal to noise ratio (i.e. enhance activity in active cells and depress activity in less active cells) in target areas. Noradrenaline transmission is suggested to be involved in e.g. attention, behavioral reorientation and is thought to function as a significance enhancer (Aston-Jones et al., 1999, Arnsten and Li, 2005). The cerebral noradrenergic transmission represents a target for a number of different psychoactive drugs, including antidepressants, antipsychotics and drugs used in the treatment of attention deficit hyperactive disorder (ADHD).

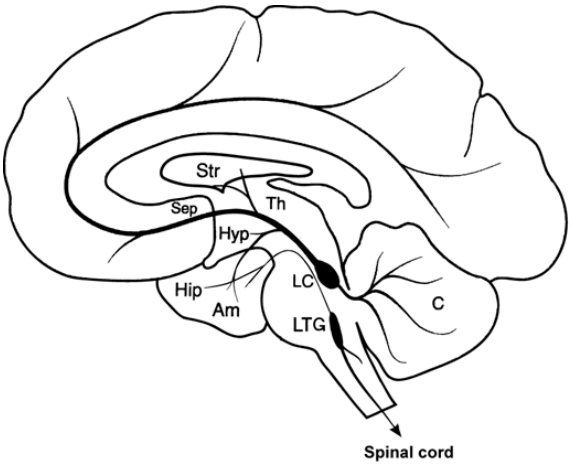

Figure 7. *Schematic drawing illustrating the noradrenergic pathways in the human brain. AM: amygdala; C: cerebellum Hip: hippocampus; Hyp: hypothalamus ;LC: locus coeruleus; LTG: lateral tegmental group; Str: striatum; sep: septum; Th: thamalus. Modified from (Rang et al., 1999).*

1.4.1. Noradrenaline synthesis and elimination

Noradrenaline is synthesized from dopamine in noradrenergic terminals by the enzyme dopamine β-hydroxylase (Brunton et al., 2011). Dopamine β-hydroxylase is bound to the vesicular membrane and, noradrenaline synthesis occurs inside the vesicles (figure 8). Noradrenaline is released via a nerve impulse-dependent mechanism and is cleared from the synaptic cleft by the NET. Noradrenaline is metabolized to its major metabolite 3-methoxy-4-hydroxyl-phentylenglycol (MHPG) by COMT and MAO.

1.4.2. Noradrenaline projections

Noradrenergic cell bodies are located in several clusters in the brain stem and can be divided into two subgroups, the LC and the lateral tegmental nuclei (Dahlström and Fuxe, 1964; figures 2 and 7). The LC projects to most of the cerebral cortex, as well as to e.g. the cerebellum, hippocampus and the amygdala. The lateral tegmental nuclei project mainly to other brain regions such as the brain stem, the hypothalamus, parts of the amygdala and the spinal cord.

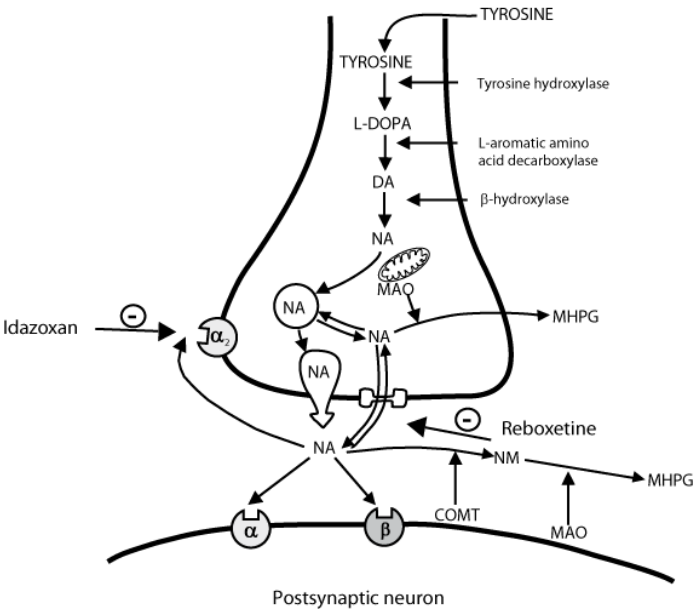

Figure 8. *Schematic drawing of a noradrenergic nerve terminal. DA; dopamine; MHPG: 3-methoxy-4-hyroxyl-phentylenglycol ; COMT: catecholamine-O-transferase; MAO: monoamine oxidase; NA: noradrenaline NM; normetanephrine. Modified from (Cooper et al., 2003).*

1.4.3. Noradrenaline receptors

There are two types of noradrenaline receptors α- and β-adrenoceptors (Bylund et al., 1994, Civantos Calzada and Aleixandre de Artinano, 2001). The α-adrenoceptors are divided into α_1- and α_2- adrenoceptors which are both widely distributed in the brain (Nicholas et al., 1996). The α_1 receptors are positively coupled to G_q and thus stimulates phospholipase C and increases IP_3 and DAG. There are three subclasses of the α_1 -adrenoceptors, the $\alpha_{1A/B/D}$. α_1-receptors are predominantly located on postsynaptic neurons e.g. on pyramidal cells of the mPFC where they co-localize with $5\text{-}HT_{2A}$ receptors and increase the excitation of the cells (Santana et al., 2013). Presynaptically located α_1-adrenoceptors have been found in e.g. NAc, where they are thought to regulate dopamine release (Mitrano et al., 2012). α_2-adrenoceptors are negatively coupled to cAMP production and thereby act inhibitory. The inhibitory function of presynaptic α_2-adrenoceptors on transmitter release was first demonstrated on central noradrenaline neurons by Andén and colleagues (Anden et al., 1970b) and, independently, on peripheral sympathetic nerves by Langer (Langer, 1970). Now it is known that α_2-adrenoceptors act as hetero- and autoreceptors, regulating noradrenergic as well as serotonergic and dopaminergic transmission (Svensson et al., 1975, Gobert et al., 1998, Devoto et al., 2001). There are three subclasses of β-adrenoceptors, $\beta_{1/2/3}$, but only $\beta_{1/2}$ are expressed in the CNS (Nicholas et al., 1996). The $\beta_{1/2}$-receptors are positively coupled to G_s activating adenylyl cyclase. β-adrenoceptors have been found to modulate neurotransmission in the mPFC and may be involved in for example memory retrieval (Ji et al., 2008, Reyes-Lopez et al., 2010).

1.5. Prefrontal cortex

The human PFC has been divided into three anatomically different regions the lateral, medial and orbital regions (Fuster, 2001). The PFC is involved in emotional behavior and cognitive processes that includes behavior, speech and reasoning, planning and executive function. In the PFC, information from external sources (sensory information) and internal sources (memories, mood) is integrated and an appropriate response is selected. The human PFC is not fully mature until early adulthood (Fuster, 2001). Patients who sustained lesions in the dorsolateral PFC (dlPFC) may display cognitive deficits such as problems with generating coherent speech, memory retrieval as well as working memory and attention deficits (Stuss and Levine, 2002). The critical importance of the PFC for working memory is supported by a plethora of animal studies. Hypofunction of the PFC is well established in schizophrenia, and is thought to contribute to the negative symptoms and cognitive deficits (c.f. 1.7). Recent studies, using imaging techniques, showed that poor activation of the dlPFC corresponded to poor cognitive performance in schizophrenic patients, but not in patients suffering from cognitive decline caused by ageing (Dreher et al., 2012). This indicates that the dlPFC dysfunction is a core deficit in schizophrenia, but not for poor cognition *per se*. The dlPFC is also implicated in emotional processing and impaired function of the dlPFC has been proposed also in depression (Savitz and Drevets, 2009). For example, hypometabolism and even reduced grey matter in the dlPFC has been observed in MDD.

The rat cerebral cortex is approximately 1000 times smaller than that of a human cortex, making a direct translation based on anatomy alone impossible (c.f. Uylings et al., 2003). The region in rat cortex that best corresponds to the human dlPFC is the rat medial PFC (mPFC; Ongur and Price, 2000, Uylings et al., 2003). This notion is based on the fact that the rat mPFC, in similarity to the human dlPFC, forms extensive reciprocal projections from e.g. the mediodorsal thalamus, receives similar neurotransmitter input (e.g. noradrenaline from the LC, serotonin from the DRN, dopamine from the VTA) and expresses similar receptors as the human dlPFC. In addition, analogous behaviors are mediated via these areas in humans and rats, respectively, such as attention, working memory and social interaction. The rat mPFC is considered to consist of four regions, medial (frontal) agranular, anterior cingulate cortex, prelimbic cortex and infralimbic cortex (Ongur and Price, 2000, Uylings et al., 2003, Hoover and Vertes, 2007). The medial agranular and the anterior cingulate cortex receive afferents from large areas of the cortex and thalamic nuclei whereas the prelimbic and infralimbic corex generally receive less cortical afferents and instead more limbic afferents (Hoover and Vertes, 2007).

1.6. Nucleus Accumbens

The NAc is a forebrain structure that makes up most of the ventral striatum. The main cell type of the NAc is the GABAergic medium spiny neurons, which express D_1 or D_2 receptors (Tritsch and Sabatini, 2012). The NAc receives dopaminergic input from the VTA, via the mesolimbic dopamine projection, and glutamatergic input from limbic regions as well as the mPFC. The NAc has been suggested to act as an interface between the motor system and the limbic system, in which motivation is translated into action (Mogenson et al., 1980). Thus, the NAc is important for a number of processes including reward, reinforcement, hedonia and motivation. The NAc can be subdivided into the shell and core compartments. The shell mainly receives input from the infralimbic subdivision of the mPFC and the core from the prelimbic subdivision. The core region is functionally related to dorsal striatum and is thought to be involved in motor function whereas the shell region is thought to be more associated with the limbic system and to be involved in motivational and emotional processes (Deutch, 1993). Studies show that clozapine and other atypical APDs preferentially increase dopamine release in the shell region, whereas the typical APD haloperidol induces dopamine release preferentially in the core (Marcus et al., 2000, Marcus et al., 2002)

1.7. Schizophrenia

Schizophrenia is a severe psychiatric disorder which affects almost all domains of the personality as well as the mental capacity and thereby severely affects the ability of an individual to function in society. The severity of the symptoms and the fact that the first symptoms usually appear in the late teens or early adulthood, i.e. periods important for e.g. education, building a career and family, contribute to the fact that the disease usually is associated with short education, low rates of employment and marriage, as well as low income (Crump et al., 2013).

The estimated lifetime prevalence of schizophrenia is approximately 0.5 to 1% (Regier et al., 1988, Carpenter and Buchanan, 1994, Goldner et al., 2002). Most patients experience their first psychotic symptoms in adolescence or early adulthood (an der Heiden and Hafner, 2000). In a majority of patients, schizophrenia develops into a chronic disease with poor outcome (Carone et al., 1991, Bromet and Fennig, 1999). As a consequence, schizophrenic patients have a reduced life expectancy of approximately 12 to 15 years mainly due somatic diseases (e.g. ischemic heart disease and cancer) but in addition, schizophrenia is associated with high risk of suicide and other causes of unnatural death (Casey et al., 2011, Crump et al., 2013). Even though some APDs are associated with severe side effects such as the metabolic syndrome, they have still been found to significantly reduce mortality in schizophrenia (Tiihonen et al., 2009, Crump et al., 2013). In addition, co-morbid diseases such as drug abuse are common and may significantly worsen the prognosis of schizophrenia (c.f. Krystal et al., 1999).

1.7.1 Symptoms of schizophrenia

The diverse symptoms of schizophrenia were first described as one disease under the name dementia praecox by the German psychiatrist Emil Kraepelin about a century ago (Kraepelin, 1919). The symptoms may vary considerably between patients and also within a single patent over time. The disease is mostly preceded by a prodromal phase, characterized by unspecific symptoms such as restlessness, anxiety, depressive and negative symptoms (see below), which can last several years before the patients experience their first psychotic episode (an der Heiden and Hafner, 2000). There is no specific diagnostic test and schizophrenia is diagnosed according to diagnostic manuals; Diagnostics and Statistical Manual of Mental Disorders (DSM-IV or the recently implemented new edition DSM-V; (American Psychiatric Association, 2000) or the International Classification of Diseases (ICD-10;WHO, 1992) and the symptoms are often divided into three broad clusters; positive symptoms, negative symptoms and cognitive deficits (Andreasen and Olsen, 1982, Gold and Harvey, 1993). Positive symptoms, sometimes referred to as psychotic symptoms, include delusions (such as thought broadcasting or communication with aliens), hallucinations (mostly auditory), formal thought disorder and catatonia. Negative symptoms include social withdrawal, flattened affect, apathy, anhedonia (inability to feel pleasure) and alogia (poverty of speech). Schizophrenia is associated with deficits in almost all cognitive domains, but with high a degree of interpersonal heterogeneity. The most characteristic cognitive impairments include deficits in working memory, attention and executive function, with less deficits found in other cognitive domains, e.g. spatial ability (Heinrichs and Zakzanis, 1998). Cognitive deficits in schizophrenia, indicated by e.g. low IQ and poor educational performance, pre-date the psychotic symptoms (Jones et al., 1994, David et al., 1997). Importantly, the severity of cognitive deficits, such as impaired verbal working memory and vigilance, predicts treatment outcome in schizophrenia to a higher degree than psychotic symptoms (Green, 1996), suggesting that treatments that effectively may ameliorate the cognitive impairments would be particularly advantageous. Cognitive impairments in schizophrenia are more stable than the psychotic symptoms, which may fluctuate considerably over time. Moreover, cognitive

deficits similar to those found in schizophrenia have been found in unaffected first-degree relatives (Snitz et al., 2006), implicating cognitive deficits as an endophenotype of the disease. In addition to impairments in higher cognitive functions, deficiencies in sensory information processing (Braff et al., 1978) and motor speed and coordination (Flashman et al., 1996) have been found associated with schizophrenia, indicating a more general neuropsychiatric deficit that may reflect a neurodevelopmental impairment.

1.7.2. Etiology of schizophrenia

The cause or causes of schizophrenia are not known, however, both genetic and environmental factors have been found to contribute. For example, having a first-degree relative with schizophrenia significantly increases the risk of developing the disease (see e.g. Lichtenstein et al., 2009).

Linkage and genome-wide association studies have found several susceptibility genes and short nucleotide polymorphisms that are associated with schizophrenia, some of which are shared with other disorders e.g. bipolar disorder. However, each gene variant seems to account for very little of the increased risk; rather it is the contribution of many gene variants that together convey an increased risk of developing schizophrenia (Harrison and Weinberger, 2005, Purcell et al., 2009, Ripke et al., 2013). Moreover, rare alleles conveying a high risk as well as *de novo* mutations may also play a role in the development of schizophrenia (for review see Doherty et al., 2012).

In addition to susceptibility genes, several environmental factors have been found to increase the risk of acquiring schizophrenia. For example, several prenatal factors such as winter birth, obstetric complications, and intrauterine influenza infections have been proposed as risk factors for schizophrenia (for review see Bromet and Fennig, 1999). Furthermore, poor socioeconomic background (Bromet and Fennig, 1999), urban living (Lewis et al., 1992), migration (Cantor-Graae and Selten, 2005) as well as drug abuse, most notably use of certain stimulants and cannabis, has been found to increase the risk of developing schizophrenia (Andreasson et al., 1987, Callaghan et al., 2012).

1.7.3. The dopamine hypothesis of schizophrenia

The first indication that the dopamine system may be involved in schizophrenia was the discovery by Arvid Carlsson that chlorpromazine and haloperidol both enhanced the turnover of catecholamines (Carlsson and Lindqvist, 1963). They proposed that the effect represented a compensatory activation of the dopamine system due to a blockade of catecholamine receptors. Later it was found that a range of clinically used APDs were indeed dopamine receptor antagonists, subsequently identified as D_2 receptor antagonists (Anden et al., 1966, Anden et al., 1970a, Creese et al., 1975, 1976, Seeman et al., 1976). It was observed that amphetamine, which enhances the release of catecholamines in the brain, may elicit or aggravate preexisting psychotic symptoms, which in turn could be blocked by APDs (Angrist et al., 1974). Moreover, L-DOPA, the precursor to dopamine, may also worsen psychotic symptoms in schizophrenic patients (Angrist et al., 1973). These findings lead to formulation of the dopamine hypothesis,

which suggests that schizophrenia is associated with an enhanced dopaminergic neurotransmission in the brain (Carlsson, 1978). Later studies demonstrated that although basal dopamine release is appears similar in patients and healthy subjects, amphetamine induces a larger dopamine release in the STR of schizophrenic patients than in healthy controls (Laruelle et al., 1996). This difference was only evident when the patients were in a psychotic state (Laruelle et al., 1999), indicating that the psychotic symptoms of schizophrenia may indeed be related to increased dopamine release. In addition to an enhanced subcortical dopaminergic transmission contributing to the positive symptoms of schizophrenia, several lines of evidence indicate that the negative symptoms may be related to impaired dopaminergic transmission in the PFC. This has led to a modified version of the dopamine hypothesis, which posits that an hyper-reactive mesolimbic dopaminergic transmission is associated with the positive symptoms of schizophrenia, whereas a hypoactive mesocortical dopamine system may largely contribute to the negative symptoms and cognitive impairments.

For example, schizophrenia is associated with hypofrontality i.e. reduced cerebral blood flow in the frontal lobes (Ingvar and Franzen, 1974) and some of the symptoms of schizophrenia resemble those observed in frontal lobe damage (Stuss and Benson, 1984). Accordingly, schizophrenic patients taken as a group perform poorly in tasks that involve the PFC, e.g. working memory tests. This is associated with a hypoactivation of the dlPFC (Dreher et al., 2012). However, some schizophrenic patients with less working memory impairment may even display a hyperactivation of the dlPFC (Callicott et al., 2000).

In contrast to the effect of D_2 blockade on the positive symptoms, D_2 receptor blockade has little effect on negative symptoms and cognitive impairments in schizophrenia and may, in fact, even worsen them (Carpenter, 1996, Saeedi et al., 2006). Interestingly, amphetamine, which exacerbates positive symptoms, may actually reduce negative symptoms and cognitive impairments in some patients (Laruelle et al., 1999, Lindenmayer et al., 2013).

Results from studies in primates show that the PFC requires an optimal level of dopamine and D_1 receptor activation for proper working memory function (Sawaguchi et al., 1988, Sawaguchi and Goldman-Rakic, 1991, Williams and Goldman-Rakic, 1995). Dopamine D_1 receptor activation display an inverted U-shape form, i.e. too little or too much dopamine in the PFC impairs working memory (Vijayraghavan et al., 2007). Interestingly, amphetamine was found to enhance activation of the PFC in schizophrenic patients and to improve cognitive performance (Daniel et al., 1991), suggesting that low cortical dopamine level contribute to the cognitive deficits in schizophrenia. The ability of clozapine to preferentially potentiate dopamine release in the mPFC is suggested to underlie its effect on cognitive impairments and negative symptoms in schizophrenia (Moghaddam and Bunney, 1990, Nomikos et al., 1994, Goldman-Rakic et al., 2004).

Alterations in prefrontal dopamine transmission have also been found in patients. Several imaging studies have observed alterations in prefrontal D_1 receptor binding in schizophrenic patients, further supporting a dysregulated dopaminergic transmission contributing to the symptoms. While Okubo and colleagues (Okubo et al., 1997) found the D_1 receptor binding to be decreased in the PFC, correlating with negative and cognitive symptoms, Abi-Dargham and colleagues found the D_1 receptor binding to be increased in the PFC (Abi-Dargham et al., 2002, Abi-Dargham et al., 2012). The higher D_1 receptor binding correlated with poor working memory in one of the studies (Abi-Dargham et al., 2002). The discrepancy between these seemingly contradictory studies may be attributed to methodological differences (for further discussion see Abi-Dargham et al., 2002). Abi-Dargham and colleagues suggest that the enhanced number of D_1 receptors may be due to a compensatory up-regulation of D_1 receptors due to decreased dopamine stimulation. This conclusion was recently substantially supported by an imaging study, which demonstrated a reduced cortical dopamine release in schizophrenic patients (Abi-Dargham, 2011).

1.7.4. Glutamate hypothesis of schizophrenia

Dopamine is not the only neurotransmitter implicated in schizophrenia. In 1959, Luby and colleagues discovered that PCP, later shown to be a non-competitive NMDA receptor antagonist, could induce a schizophrenia-like state which was almost indistinguishable from schizophrenia (Luby et al., 1959, Javitt and Zukin, 1991). In similarity, ketamine, also a non-competitive NMDA receptor antagonist may induce positive and negative symptoms as well as cognitive impairments in healthy volunteers that are similar to those observed in schizophrenia (Krystal et al., 1994). Moreover, low, sub-dissociative doses of ketamine have been found specifically to impair verbal working memory in healthy volunteers, a common cognitive deficit in schizophrenia (Honey et al., 2003). Moreover, PCP, and other NMDA receptor antagonists have been found to worsen symptoms in schizophrenic patients (Luby et al., 1959, Lahti et al., 1995, Malhotra et al., 1997).

Several of the risk genes associated with schizophrenia have been shown to be linked to NMDA receptor-mediated signaling (e.g. DISC1 and dysbindin) and could contribute to an aberrant NMDA receptor-mediated transmission (Snyder and Gao, 2013). Genetically modified mice with reduced expression of the NMDA receptor subunit NR1 display behavioral abnormalities analogous to schizophrenia for example deficits in social interaction (Mohn et al., 1999).

Other data supporting the involvement of NMDA-receptor abnormalities in schizophrenia are derived from post mortem studies indicating alterations in the expression of several glutamatergic receptors in schizophrenia e.g. the NMDA receptor subunits NR2A and NR1 as well as associated postsynaptic proteins in the PFC (Dracheva et al., 2001, Kristiansen et al., 2007, Beneyto and Meador-Woodruff, 2008). More recently, alterations in the post-translational modifications of kainate and AMPA glutamatergic receptors have been identified. These modifications are thought to affect

translocation of the receptors (Tucholski et al., 2013a, Tucholski et al., 2013b), and may thereby also contribute to aberrant glutamatergic neurotransmission in schizophrenia.

In addition to various alterations in the expression of glutamate receptors, patients suffering from schizophrenia have low cerebrospinal fluid (CSF) levels of the NMDA receptor co-agonist D-serine, an observation supported by findings from post mortem and genetic studies, suggesting that dysregulation of D-serine levels may contribute to NMDA receptor hypofunction (see Labrie et al., 2012). Thus, these observations propose that NMDA receptor hypofunction contributes to the symptoms of schizophrenia (Javitt and Zukin, 1991, Krystal et al., 1994).

The involvement of both dopamine and glutamate in schizophrenia is not surprising since there is substantial interaction between the dopaminergic and glutamatergic systems in the brain and NMDA receptor antagonists have been shown to increase dopamine turnover in healthy volunteers (Krystal et al., 1994), affect dopamine cell firing rate and firing patterns (Murase et al., 1993b) and increase dopamine output in both cortical and subcortical areas of the brain see e.g. (Mathe et al., 1999). Dopamine D_1 receptors and NMDA receptors interact on pyramidal cells in the PFC, a mechanism important for cognition (see 1.2.4). Moreover, ketamine-abuse has been reported to increase the number of D_1 receptors in the dorsolateral PFC (dlPFC; Narendran et al., 2005), in similarity to findings in schizophrenic patients (see above).

In addition to dopamine and glutamate, also other neurotransmitters have been implicated in schizophrenia. For example, lysergic acid diethylamide (LSD) and other drugs acting as 5-HT_2 agonists cause altered perception and hallucinations, implicating serotonin in schizophrenia (for review see e.g. Aghajanian and Marek, 2000). 5-HT_2 agonists suppress firing of serotonergic neurons in the raphe nuclei and may in addition increase glutamate release in the PFC. These effects have been shown to generate decreased synchronization of the activity of pyramidal cells in the PFC, which has been suggested to mediate hallucinations (Aghajanian and Marek, 2000). However, LSD and other 5-HT_2 agonists mainly induce visual hallucinations, which are rarely observed in schizophrenia, and produce symptoms reminiscent of negative symptoms or cognitive impairments to a minor extent indicating that deficits in serotonergic transmission alone cannot explain the full symptomatology of schizophrenia. Moreover, increased levels of kynurenic acid, an endogenous substance derived from astrocytes, with antagonistic properties at the α_7-nicotinic receptor and NMDA receptor has been found in the CSF of patients suffering from schizophrenia (Erhardt et al., 2001) although the pathophysiological significance of these findings remains to be fully understood.

1.8. Antipsychotic drugs

Before the introduction of APDs, the treatment of schizophrenic patients was limited to unspecific pharmacological treatments (such as opium or chloral hydrate) or to therapies, such as electroconvulsive therapy or even insulin shock. These treatments had in common that they had no sustained effect and as a result, patients often required frequent or life-long hospitalization.

1.8.1. Typical antipsychotic drugs (first generation antipsychotic drugs)

The first drug to show a specific antipsychotic effect was chlorpromazine, a drug that was first synthesized in 1950. Chlorpromazine was originally developed as an antihistaminergic drug and was initially used to reduce shock after surgery. In 1952, chlorpromazine was found to alleviate symptoms of schizophrenia and mania (for review see (Lopez-Munoz et al., 2005), a finding that revolutionized psychiatric care and reduced the number of patients requiring hospitalization dramatically (c.f. Carpenter and Davis, 2012). At approximately the same time an extract from the plant Rauwolfia serpentina, which was used for the treatment of hypertension, containing among other substances reserpine and yohimbine, was also found to possess an antipsychotic action (see e.g. Kline, 1954). These findings spurred the search for other APDs and subsequently, in the same decade, Paul Janssen and colleagues developed haloperidol (Divry et al., 1958, Granger and Albu, 2005) a drug that was a much more selective dopamine receptor antagonist than chlorpromazine. These drugs (except reserpine), initially called major tranquilizers or neuroleptics, are now often referred to as typical APDs or first generation APDs and are still frequently used in the treatment of schizophrenia.

The common mechanism of action of typical APDs is blockade of the D_2 family of receptors, however affinity for other receptors may also contribute to the antipsychotic effect. To produce an antipsychotic effect, typical APD treatment must produce approximately 70% D_2 receptor occupancy in STR (Farde et al., 1988a, Farde et al., 1992). Unfortunately, a high degree of D_2 blockade i.e. above 80% occupancy increases substantially the risk of EPS such as akathisia (inner restlessness and discomfort), dystonia (sustained involuntary muscle contractions), parkinsonism (tremor, hypokinesia and rigidity) and tardive dyskinesia (involuntary movement of e.g. the lips, tongue and extremities). Moreover, the typical APDs may increase prolactin levels, inducing endocrine side effects such as galactorrhea.

Although typical APDs are generally effective in ameliorating positive symptoms of schizophrenia, they have less effect on negative and cognitive symptoms. In fact, treatment with D_2 receptor antagonists, e.g. haloperidol, may even worsen negative symptoms, have a negative impact on mood and induce cognitive deficits in healthy volunteers (Carpenter, 1996, Saeedi et al., 2006).

1.8.2. Atypical antipsychotic drugs (second generation antipsychotic drugs)

In the 1950's another APD that would also revolutionize the treatment of schizophrenia was first developed, namely clozapine. Based on its structure clozapine was initially thought to be an antidepressant drug, although subsequent studies in the mid 60's by Hippius demonstrated its antipsychotic effects. However, in contrast to e.g. haloperidol, clozapine treatment was devoid of EPS in patients and did not induce catalepsy in laboratory animals; at that time thought prerequisite for antipsychotic activity. Because of this property, clozapine was considered an atypical APD compared to chlorpromazine and haloperidol (for review see Hippius, 1989, 1999). The discovery of

the atypical profile of clozapine has inspired the search for other atypical, or second generation APDs, with similar structure e.g. olanzapine and quetiapine.

Clozapine has since then been found superior to both first generation and other second generation APDs in treatment-resistant schizophrenia (Kane et al., 1988, Taylor and Duncan-McConnell, 2000, McEvoy et al., 2006, Swartz et al., 2008). Furthermore, clozapine has been found to reduce suicidal behavior in schizophrenia and schizoaffective disorder (Meltzer et al., 2003, Hennen and Baldessarini, 2005). Clozapine exerts its antipsychotic effect at a low striatal D_2 occupancy (~45%) compared to typical APDs which generally require almost 70% occupancy to exert an antipsychotic effect. As a consequence, clozapine has a very low risk of EPS (Farde et al., 1992, Nordstrom et al., 1995, Kessler et al., 2006b). Clozapine has higher affinity for several 5-HT receptors (including the 5-HT_2 receptor) and the α_2-adrenoceptor than for the D_2 receptors (Schotte et al., 1996, Marcus et al., 2005). These properties have been proposed to contribute to the superior efficacy of clozapine in schizophrenia (Meltzer et al., 1989, Nutt, 1994, Hertel et al., 1999a, Svensson, 2003). Clozapine has also been shown to ameliorate negative symptoms and cognitive impairments in schizophrenia (Meltzer and McGurk, 1999, Leucht et al., 2009). Unfortunately, clozapine treatment may be associated with several severe side effects and, in fact, clozapine was even withdrawn from the market because of associated agranulocytosis (Idanpaan-Heikkila et al., 1977). The drug was subsequently reintroduced in 1990 because of its superior efficacy (Kane et al., 1988), although patients receiving clozapine require regular hematological monitoring. In addition, clozapine treatment is often associated with weight gain and the metabolic syndrome (Mitchell et al., 2013). Despite these severe side effects and the fact that clozapine is mostly prescribed to treatment-resistant patients, the use of clozapine is associated with the lowest mortality rates compared with all other APDs investigated (Tiihonen et al., 2009).
The effect of clozapine on negative symptoms and cognitive impairments is thought to be related to the increased dopamine output in the PFC (Imperato and Angelucci, 1989, Moghaddam and Bunney, 1990, Nomikos et al., 1994). Subsequently, also other atypical APDs have been found to increase dopamine output in the PFC (see e.g. (Li et al., 1998). The mechanism by which atypical APDs induce the cortical dopamine release is not entirely clear, although it may involve a blockade of 5-HT_{2A} and D_2 receptors and indirect activation of 5-HT_{1A} (Ichikawa et al., 2001, Ichikawa et al., 2002, Liegeois et al., 2002). In addition to the blockade of 5-HT_{2A} receptors, clozapine induces dopamine release in the PFC by blockade of α_2-adrenoceptors (see Hertel et al., 1999a, Devoto et al., 2003). Affinity for other receptors may also contribute to the antipsychotic effect, for example, clozapine acts as partial agonist at D_1 and 5-HT_{1A} receptor (Salmi et al., 1994a, Newman-Tancredi et al., 1996).

In addition to the increased cortical dopamine release, clozapine, as well as other atypical APDs, has been found to facilitate both NMDA-induced currents and EPSPs in pyramidal cells in cortical slices, an effect which may also contribute to the superior effect of clozapine on negative symptoms and cognitive deficits in schizophrenia

(Arvanov et al., 1997, Ninan et al., 2003b). The facilitation of NMDA-induced currents and EPSPs induced by APDs, at is least in part, dependent on activation of the dopamine D_1 receptors (Chen and Yang, 2002, Ninan and Wang, 2003, Jardemark et al., 2010). The effect of clozapine has been found to be mediated by PKA and protein kinase C (PKC) as well as the calcium/calmoduline-dependent kinase II (CaMKII) (Jardemark et al., 2003, Ninan et al., 2003a, Wittmann et al., 2005). Interestingly, clozapine reduced the binding of a selective tracer that binds to the PCP site of the NMDA receptor, indicating that clozapine indeed activates NMDA receptor-mediated transmission in patients (Bressan et al., 2005). In contrast to the effects on NMDA receptor-mediated transmission, atypical APDs such as olanzapine or clozapine have not been found to affect AMPA receptor-mediated transmission in the mPFC (Arvanov et al., 1997, Ninan et al., 2003b).

Preclinical studies have shown that, in addition to its importance in cognition, dopamine transmission in the mPFC, especially D_1 receptor activation, modulates dopamine release in the NAc and subcortically derived D_2 receptor mediated behaviors (Pycock et al., 1980, Vezina et al., 1991, Scornaiencki et al., 2009). Thus, dopamine in the cortex acting on D_1 receptors may contribute to regulate dopamine-mediated behaviors controlled by subcortical dopamine pathways.

Low doses of L-DOPA given with APDs may augment the effect of APDs in schizophrenia (Jaskiw and Popli, 2004). L-DOPA treatment increases dopamine levels more in the PFC that in the NAc (Loeffler et al., 1998) and when combined with a low sub-effective dose of raclopride, L-DOPA treatment potentiates the suppression of CAR behavior and, in parallel, induces a preferential increase in dopamine output in the mPFC (Eltayb et al., 2005) supporting the notion that enhanced cortical dopamine levels *per se* contributes to an antipsychotic effect.

A number of atypical APDs have been developed since the discovery of clozapine. There is considerable diversity among them with regard to their receptor binding profiles as well as clinical efficacy. As a group, atypical APDs have a broader receptor binding profile than typical APDs, with affinity for a wide range of receptors which contributes to their antipsychotic effect. Most atypical APDs have high affinity for several serotonergic receptors, most notably 5-$HT_{2A/C}$ receptors.

For example, olanzapine is an atypical APD that is structurally similar to clozapine and has, in similarity to clozapine, higher affinity for e.g. 5-HT_{2A}, 5-HT_{2C}, 5-HT_6 and the histamine H_1 receptor than for the D_2 receptor (Schotte et al., 1996). However, in contrast to clozapine, olanzapine lacks high affinity for the α_2-adrenoceptor (Shahid et al., 2009). Olanzapine has been found effective against positive and negative symptoms in schizophrenia but unfortunately, olanzapine treatment is often associated with side effects such as the metabolic syndrome and weight gain (Beasley et al., 1997). Positron emission tomography (PET) studies in patients, show that olanzapine treatment produce a very high occupancy on the 5-HT_2 receptors (>90% even at 5 mg/day) and a D_2 occupancy which was generally higher (i.e. 55 - 88%) than that obtained with clozapine

(Nordstrom et al., 1995, Kapur et al., 1998) and, in fact, similar to that observed in patients receiving typical APDs (Zipursky et al., 2005).

Interestingly, the atypical APD quetiapine is effective at lower D_2 receptor occupancy than olanzapine and similar to that obtained with clozapine treatment (Borison et al., 1996, Arvanitis and Miller, 1997, Kessler et al., 2006b). Consequently, quetiapine treatment is associated with very low risk of EPS or increased prolactin levels (Borison et al., 1996, Arvanitis and Miller, 1997). In addition to its effect in schizophrenia, quetiapine is effective as monotherapy in bipolar disorder and MDD in approximately the same dose range (Calabrese et al., 2005, Cutler et al., 2009). Quetiapine has been found to possess higher affinity for 5-HT_{2A}, 5-HT_{1A}, α-adrenoceptors and H_1 receptors than the D_2 receptors (Schotte et al., 1996). In a clinical study investigating the effects of atypical APDs in schizophrenia, quetiapine was found to be more efficacious compared to other atypical APDs in relieving certain neurocognitive deficits in schizophrenia (Riedel et al., 2010). However, it should be noted that clozapine was not included in the study.

One of the newest atypical APDs is asenapine. Asenapine has a multi-receptor binding profile and has higher affinity for several receptors (5-HT_{2A}, 5-HT_{2b}, 5-HT_{2c}, 5-HT_6 and 5-HT_7, α_{2B} and D_3) than for the D_2 receptor (Shahid et al., 2009). In clinical studies, asenapine was found efficatious in reducing positve as well as negative symtoms of schizophrenia, with little metabolic disturbances or weight gain (Potkin et al., 2007, Schoemaker et al., 2010). In additon, asenapine has been found effective in mania as well as to reduce depressive symtoms in mixed states associated with bipolar disorder (Vita et al., 2013). Preclincal studies propose that asenapine may be effective in ameliorating cognitive deficts associated with schizophrenia and that this effect may be mediated via D_1 receptor activation in the mPFC (Jardemark et al., 2010, Snigdha et al., 2011, Elsworth et al., 2012).

The drug raclopride was originally developed as an APD and is a highly selective $D_{2/3}$ receptor antagonist. Raclopride was found to produce an antipsychotic in clinical studies (Farde et al., 1988b). However, raclopride is not used clinically but is widely used as pharmacological tool in e.g. PET studies (then as 11C-raclopride) (Farde et al., 1985, Kohler et al., 1985).

All the above mentioned APDs have shown antipsychotic-like effect in preclinical models (Hillegaart and Ahlenius, 1987, Moore et al., 1992, Wadenberg et al., 2001, Franberg et al., 2008). Using microdialysis, the atypical APDs olanzapine, quetiapine and asenapine have all been shown to increase dopamine and noradrenaline output in the rat PFC and to a lesser extent dopamine output in the NAc (Li et al., 1998, Ichikawa et al., 2002, Franberg et al., 2008, Franberg et al., 2009, Yamamura et al., 2009). Raclopride, having a typical APD profile, preferentially enhances dopamine output in the NAc, compared to the mPFC (Hertel et al., 1999a), an effect similar to that of haloperidol. Interestingly, asenapine has also been found to increase serotonin output in the mPFC, an effect not obtained by olanzapine and quetiapine (Li et al., 1998,

Ichikawa et al., 2002, Franberg et al., 2009, Yamamura et al., 2009). In similarity with clozapine, the atypical APDs olanzapine, quetiapine and asenapine all have been found to facilitate NMDA receptor-mediated currents in pyramidal cells, using intracellular recordings *in vitro* (Ninan et al., 2003b, Franberg et al., 2008, Jardemark et al., 2010). Raclopride however, does not affect NMDA-induced currents in pyramidal cells, in line with its typical profile (Jardemark et al., 2009).

The atypical APD aripiprazole acts as a partial agonist at the D_2 receptor, and is sometimes called a third generation APD. Partial agonism at the D_2 receptor is thought to stabilize rather than to block dopaminergic transmission (Keck and McElroy, 2003). In addition to D_2 partial agonism, aripiprazole is a partial 5-HT_{1A} agonist and a 5-HT_{2A} antagonist. Aripiprazole treatment is associated with low risk for EPS and prolactin increase as well as weight gain (Keck and McElroy, 2003).

1.8.3. Adjunctive antidepressants added to APDs in schizophrenia

In a series of studies, Tiihonen and colleagues have shown that addition of the antidepressant drug mirtazapine to typical APDs may improve positive and negative symptoms, as well as cognitive deficits and depressive symptoms in schizophrenia (Joffe et al., 2009, Stenberg et al., 2010, Terevnikov et al., 2010, Stenberg et al., 2011, Terevnikov et al., 2011). Mirtazapine is an $\alpha_{2A/C}$-adrenoceptor and at 5-HT_{2C} receptor antagonist, which preferentially increases dopamine and noradrenaline output in the frontal cortex with little effect in the NAc (Millan et al., 2000). Thus, addition of mirtazapine to low doses of APD generates a binding profile reminiscent of clozapine (c.f. 1.8.2). Similarly, addition of selective α_2-adrenoceptor antagonists (e.g. idazoxan) to typical APD may reduce both positive and negative symptoms of schizophrenia (Litman et al., 1996, Hecht and Landy, 2012).

1.9. Bipolar disorder

Bipolar disorder, sometimes called manic-depressive disorder, is a disease characterized by shorter manic (bipolar depressive disorder type I) or hypo-manic episodes (bipolar disorder type II) followed by longer euthymic and/or depressive episodes (Judd et al., 2002). Cyclothymic disorder and bipolar disorder not otherwise specified are also considered to belong to the bipolar disorder spectrum. Depression is more prevalent than mania and it is estimated that bipolar patients experience depressive symptoms approximately 1/3 of the time (Judd et al., 2002). Moreover, patients with bipolar disorder also experience subsyndromal depressive symptoms which are associated with impairment at work and in social life (Altshuler et al., 2006). The lifetime prevalence of bipolar disorder is estimated to approximately 1% (Regier et al., 1988), but is considered by some to be substantially higher (Akiskal et al., 2000) mainly due to the fact that many patients with an MDD diagnosis experience shorter episodes of mania or hypomania without receiving a bipolar diagnosis. A recent longitudinal study found that approximately one third of the patients that had an MDD diagnosis later receive a bipolar diagnosis, a finding that may explain treatment-resistance in some MDD patients (Dudek et al., 2013).

The manic episodes are characterized by periods of elevated mood, irritability, reduced need for sleep and may include delusive symptoms such as grandiose delusions and florid religious beliefs. Some patients may also exhibit psychotic symptoms resembling those seen in schizophrenia. Manic episodes may be experienced as positive by the patient, however, their irresponsible behavior most often causes conflicts with family and colleagues and, consequently, bipolar patients in a manic state may require hospitalization, often against their will. The risk of suicide amongst bipolar patients is very high, approximately 20 times higher than in the general population (Tondo et al., 2003).

Cognitive functions such as executive function, working memory and attention is impaired in bipolar disorder (Goldberg and Chengappa, 2009) although to a lesser extent than in schizophrenia (Daban et al., 2006). Interestingly, cognitive function is impaired not only in manic or depressed states but also in the euthymic state (Martinez-Aran et al., 2004) and is apparent also in first degree relatives (Ferrier et al., 2004), indicating that impaired cognition is a trait for bipolar disorder. Moreover, in bipolar disorder, impaired cognition is associated with poor occupational functioning (Martinez-Aran et al., 2004).

1.9.1. Etiology of bipolar disorder

The etiology of bipolar disorder is not fully understood. In similarity with schizophrenia, there is a substantial genetic contribution also to bipolar disorder, some of which is shared with schizophrenia (Lichtenstein et al., 2009, Craddock and Sklar, 2013).

Environmental factors such as obstetric complications or winter-spring birth and parental loss are suggested to contribute to the risk of developing bipolar disorder, however there are discrepancies between studies (for review see Tsuchiya et al., 2003). Moreover, stressful life events, altered circadian rhythm, childbirth and use of antidepressants may precipitate a manic episode in bipolar patients (Proudfoot et al., 2011).

Imaging studies of anatomical or functional brain alterations in bipolar disorder have largely yielded inconsistent results (see e.g. Nery et al., 2013), however, in similarity to schizophrenia, bipolar disorder has been associated with an altered expression of glutamate receptors in several brain areas including the PFC (Beneyto et al., 2007, Beneyto and Meador-Woodruff, 2008).

1.9.2. Treatment of bipolar disorder

Several different types of drugs are used in the treatment of bipolar disorder, partly depending on in which state of the disease the patient is. Lithium is effective as a mood-stabilizing drug in preventing conversion to mania or depression (Cade, 1949, Geddes et al., 2004). Lithium is also effective in reducing suicide in mood disorders (Cipriani et al., 2005). The mechanism of action of lithium is complex and not fully understood and may include effects on both neurotransmitter release and intracellular processes (Malhi et al., 2013). Although generally effective, lithium has a narrow therapeutic interval and

may induce hypothyroidism and affect renal function (McKnight et al., 2012). In addition antiepileptic drugs are used as mood stabilizers, however, the efficacy differs between drugs (Cipriani et al., 2011, Geddes and Miklowitz, 2013). Although lithium and the antiepileptic drugs may alleviate an acute manic episode, recent data suggests that APDs such as haloperidol or risperidone have a better effect on manic symptoms than the mood stabilizers (Cipriani et al., 2011).

Monotherapy with antidepressant drugs such as SSRIs seems to have limited effects on bipolar depression (Sidor and MacQueen, 2012). Atypical APDs are often combined with antidepressants generating a mood stabilizing effect, and thus prevent conversion to mania or depression. Interestingly, such combinations have also been found to produce an enhanced antidepressant effect in MDD and bipolar depression, with a rapid onset (see 1.11.1).

The atypical APD quetiapine has gained widespread use in bipolar disorder and has been found effective in ameliorating both manic (Cipriani et al., 2011) and depressive (McElroy et al., 2010, Young et al., 2010) episodes as well as to increase the time to relapse of depressive events (Young et al., 2012). In a recent study investigating the efficacy of different drugs used to treat mania and depression, quetiapine was found to be almost equally effective in treating mania and depression (Popovic et al., 2012).

1.10. Major Depressive Disorder

Depression is an affective disorder characterized by periods of low mood interchanged with periods of euthymia. Depression is very common and twice as common in women as in men, with an estimated 12 month prevalence of approximately 7 % (Kessler et al., 2003, Wittchen et al., 2011) and lifetime prevalence is estimated to approximately 15 to 20 % (Kessler et al., 2003, Kessler et al., 2005). Co-morbid disorders, such as anxiety, substance use and impulse control disorder are very common and correlates with the severity of depressive symptoms (Kessler et al., 2003). Recent figures shows that depression affects 30 million people yearly in the European Union alone, and of all mental and neurological disorders depression is associated with the highest burden of disease (measured as disability-adjusted life years, DALYs) in Europe (Wittchen et al., 2011). Due to the high prevalence and the severity of symptoms, mood disorders, i.e. MDD and bipolar disorder, leads to the highest costs for society of all disorders of the brain (Gustavsson et al., 2011), costs which are mainly accounted for by indirect costs, e.g. absence from work and low productivity (Kessler et al., 2003, Kessler et al., 2006a, Gustavsson et al., 2011). Another severe consequence of depression is an increased risk of suicide. Co-morbid disorders and other risk factors (e.g. severity of the depression, anxiety disorder and drug use) significantly increase the risk of suicide in depression (Hawton et al., 2013).

Like schizophrenia and bipolar disorder, depression is diagnosed by a clinical evaluation according to DSM-IV or ICD-10. The symptoms of depression are diverse and to be diagnosed with a depressive episode according to DSM-IV, one of the two cardinal symptoms must be fulfilled; either depressed mood most of the day or

diminished interest or pleasure in all or most activities for at least two weeks. In addition, five of the following symptoms are required; unintentional weight gain or loss, hypersomnia or insomnia (early morning awakenings are very common), psychomotor agitation or retardation noticed by others, feelings of worthlessness or excessive guilt, fatigue or loss of energy, diminished ability to think or concentrate or indeciveness, recurrent thoughts of death or suicide. Given the diversity of symptoms, it is possible for two depressed patients not to share a single symptom further illustrating the heterogeneity of MDD.

1.10.1. Etiology of major depressive disorder

The etiology of depression remains to be fully understood. The heritability of MDD has been estimated to 40% (Kendler et al., 2006), which is lower than for schizophrenia and bipolar disorder. Risk genes have been found but the results have been difficult to replicate and seem to convey little of the increased risk of MDD (Shyn and Hamilton, 2010). One explanation for difficulties in finding candidate genes is that there seems to be a considerable gene x environment interaction for the risk of developing MDD (see e.g. Caspi et al., 2003).

MDD is associated with cognitive impairment, in domains such as working memory, emotional processing and attention, and these deficits may persist even after remission (Taylor Tavares et al., 2003, Preiss et al., 2009, Bora et al., 2012). Antidepressant drugs usually do not affect dopaminergic transmission which may have bearing on the relative lack of efficacy of these drugs, since there are several indications for an impaired dopamine system in MDD, especially for symptoms such as cognitive deficits, reduced drive and anhedonia (Nestler and Carlezon, 2006, Dunlop and Nemeroff, 2007).

1.10.2. Hypotheses of depression

The first drugs found to have antidepressant effect were iproniazid and imipramine, which were discovered by serendipity in the 1950's. At the time, the mechanism conveying the antidepressant effect was unknown but both drugs were found to increase the availability of monoamines in brain; iproniazid by inhibiting the enzyme monoamine oxidase (MAO) and imipramine by inhibiting the reuptake of monoamines. Based on the mechanism of action of these drugs and the fact that reserpine, a drug which reduces monoamine levels may induce depressive symptoms, it was hypothesized that depression was caused by a deficiency in monoamines, in particular noradrenaline, in the brain (see e.g. Schildkraut, 1965). Since then, many antidepressant drugs with different mechanism of action have been developed that all have in common that they enhance monoaminergic transmission in the brain. However, although augmented monoaminergic transmission may ameliorate depressive symptoms, it has been difficult to actually demonstrate reduced levels of monoamines in depressed patients, indicating that it may not be a monoamine deficiency *per se* that causes depression. Although, antidepressant drugs increase monoamine levels within hours after administration, the antidepressant response is usually delayed and patients may require treatment for weeks to months to be fully effective (Trivedi et al., 2006). One explanation to the delayed effect may be that the initial increase in monoamine levels is attenuated by inhibitory

autoreceptors (e.g. α_2 and 5-HT_{1A}) which desensitize over time allowing for the full antidepressant response (Svensson and Usdin, 1978, see Nutt, 2002). Moreover, studies in animals have shown that repeated dosage of antidepressants increases cortical plasticity (Maya Vetencourt et al., 2008) and may induce neurogenesis in the hippocampus of rodents (Malberg et al., 2000), effects which if existing in humans might contribute to explain the delayed antidepressant effect. In contrast, in a series of experiments Harmer and colleagues have shown that antidepressant drugs reduce a negative bias in emotional processing associated with MDD (Harmer, 2008). This effect is already observable within a few hours of drug administration and thus precedes the effects on mood and the authors suggest that antidepressant drugs do not enhance mood *per se* but rather affect the emotional processing which subsequently also reduces depressive symptoms.

Stress is a major risk factor for developing depression and chronic stress has been found to affect the morphology of pyramidal cells in the rat cortex. Repeated stress may induce atrophy of the dendrite arbor of layer V pyramidal cells of the rat mPFC (Liu and Aghajanian, 2008). The stress-induced atrophy has biological consequences as it reduces the excitatory input to the pyramidal cells. Indeed, MDD has been found to be associated with a dysregulated hypothalamic-pituitary-adrenal (HPA) axis, and stressful life-events is associated with the onset of depression (Kendler et al., 1995, Krishnan and Nestler, 2010). The dysregulated HPA axis is suggested to contribute to the observed neuronal atrophy as well as the symptomatology of MDD. However, hypercortisolemia is mostly found in severely depressed patients requiring hospital care and a subset of depressed patients actually display hypocortisolemi (Krishnan and Nestler, 2010).

Depression is associated with dysfunction in brain networks that regulate mood and emotion, and both imaging and post-mortem studies show a reduced gray matter volume and altered activity in several areas of the brain, including sub-divisions of the PFC, cingulate cortex, hippocampus and the ventral striatum, although some conflicting results have been obtained (Drevets et al., 2008). In the PFC, post-mortem studies have demonstrated a reduced size of neurons and number of glia cells, alterations that seem to be specific for depression (Rajkowska et al., 1999). A recent study found a reduced number of synapses and expression of several genes associated with synapse function in the dlPFC of patients suffering from MDD (Kang et al., 2012). Moreover, a reduced prefrontal expression of NR1, NR2A, NR2B, mGluR5 and PSD-95 (Beneyto and Meador-Woodruff, 2008, Feyissa et al., 2009, Deschwanden et al., 2011) as well as increased expression of mGluR2/3 receptors (Feyissa et al., 2010) has also been observed in depressed patients.

1.11. Antidepressant drugs

Imipramine was the first in what became a whole class of drugs called tricyclic antidepressants, which received the name because of their structure. The tricyclic drugs are effective in depression (Kuhn, 1958), however they have affinity for e.g. histaminergic and cholinergic receptors, and may cause serious side effects, including QT prolongation and cardiac death. Such side effects made the tricyclics far from

optimal in the treatment of depression, and therefore more selective reuptake inhibitors were developed.

Since the introduction of the first SSRI, zimelidine around 1980, several other SSRIs have been developed and have now become available (e.g. sertraline, fluoxetine and citalopram). SSRIs increase synaptic levels of serotonin by inhibiting the SERT. Numerous clinical studies have shown a significant antidepressant effect of the SSRIs (see e.g. Trivedi et al., 2006) and the increased use of SSRIs has been found to correlate well with the reduction in suicide rates observed during the last 20 years (Gibbons et al., 2005). SSRIs are associated with fewer and much less serious side effects than the tricyclic drugs and are now widely used and are the first line treatment for depression. However, the efficacy of SSRIs is less than optimal. In similarity with TCAs, SSRIs have a delayed onset of the antidepressant effect (Trivedi et al., 2006) and, importantly only about one third achieve full remission. In fact, significant symptom relief is only achieved in approximately 50% of patients treated with SSRIs (Trivedi et al., 2006).

The first SSRI that gained worldwide use is fluoxetine (Wong et al., 2005). In clinical trials, fluoxetine was found as effective as imipramine but with less side effect (Stark and Hardison, 1985). In addition to MDD, fluoxetine and other SSRIs has been found effective in other psychiatric disorders e.g. obsessive compulsive disorder and anxiety disorders (Wong et al., 2005). Preclinical studies have shown fluoxetine to increase extracellular serotonin levels in several brain regions and to be effective in animal models of depression (see Wong et al., 1995 and references therein). In a study using intracellular recordings, fluoxetine was found to increase NMDA-induced currents in pyramidal cells of the rat mPFC at 1 μM, but not at 200 nM (Arvanov et al., 1997). Another commonly used SSRI is escitalopram which is the the S-enatiomer of citalopram (which is a a racemate containing both R- and S-enantiomers) with high selectivity for the SERT. Experimetnal data indicates that the R-enantiomer of citalopram antagonizes some of the effects S-enantiomer (see e.g. Sanchez, 2006, Schilström et al., 2011). Escitalopram shows a potent effect in animal models predictive of antidepressant activity and increases serotonin output in the mPFC (Sanchez et al., 2003, Pehrson et al., 2013). Moreover, escitalopram facilitates NMDA receptor-mediated transmission in pyramidal cells of the rat mPFC *in vitro* and appaers to possess a cognitive enhancing action in experimental animals (Schilstrom et al., 2011). Escitalopram is seems to generate an enhanced antidepressant activity compared with other SSRIs, (including citalopram) with a faster onset of action (Montgomery et al., 2007, Montgomery and Moller, 2009). Escitalopram has also been found to alleviate certain cognitive imparments in depressed patients (Wroolie et al., 2006, Herrera-Guzman et al., 2009).

In addition to SSRIs, a selective noradrenaline reuptake inhibitor (NRI), reboxetine has been developed for depression. Early clinical studies indicated that reboxetine may be as effective as SSRIs in depression and was found to increase drive, cognition and social functioning in depressed patients, although with different side effect profile when compared with SSRIs (Montgomery, 1997, Schatzberg, 2000, Ferguson et al., 2003). In

preclinical studies, reboxetine has been found to possess an antidepressant-like effect, improve measures of cognitive function, increase dopamine and noradrenaline output in mPFC and the hippocampus as well as to increase burst firing of dopaminergic cell bodies within the VTA (Sacchetti et al., 1999, Wong et al., 2000, Linner et al., 2001, Borgkvist et al., 2011, De Bundel et al., 2013). Significantly, atomoxetine, an NRI used in attention deficit hyperactive disorder (ADHD) has been found to enhance cognition also in healthy volunteers (Chamberlain et al., 2006).

Several other antidepressant drugs acting on the reuptake of monoamines have been developed, such as bupropion, a dopamine and noradrenaline reuptake inhibitor (Thase et al., 2005). Venlafaxine and duloxetine are serotonin and noradrenaline reuptake inhibitors (Golden and Nicholas, 2000, Mallinckrodt et al., 2007). In addition to reuptake inhibitors, there are several other drugs that enhance monoaminergic transmission which can be used in depression, e.g. the reversible MAO-A inhibitor moclobemide (Shulman et al., 2013) and mirtazapine, which is an α_2 and 5-HT_{2C} receptor antagonist (Millan et al., 2000).

1.11.1. Rapidly acting antidepressant drugs

In 1990, Trullas and Skolnick reported that NMDA receptor antagonists possess antidepressant-like activity in preclinical studies (Trullas and Skolnick, 1990). An antidepressant effect of the non-competitive NMDA receptor antagonist ketamine was subsequently demonstrated in patients by Berman and colleagues (Berman et al., 2000). Moreover, the antidepressant effect had a very rapid onset (within hours) and was found to be sustained over several days after a single administration, despite the short half-life of ketamine (Clements et al., 1982). This original study has since been replicated and extended several times. For example, Zarate and colleagues showed that the antidepressant effect of ketamine at a single dose may last up to 2 weeks and that ketamine is effective also in bipolar depression and may reduce suicidal ideation (Zarate et al., 2006, Zarate et al., 2012). However, the use of ketamine is limited by side effects such as abuse liability and, as previously mentioned, since ketamine may induce psychotic symptoms (Krystal et al., 1994).

The robust antidepressant-like effect of ketamine observed also in preclinical models has been shown to be dependent on activation on AMPA receptors in the mPFC as well as activation of the mammalian target of rapamycin (mTOR) signaling pathway (Maeng et al., 2008, Li et al., 2010). Ketamine was found to induce an increased in synaptic proteins, e.g. GluR1, growth of dendritic spines and enhanced glutamatergic transmission in the mPFC, including increased release of glutamate in the mPFC of awake rats following acute administration of ketamine (Moghaddam et al., 1997). Interestingly, clinical studies show that a single intravenous dose of the muscarinic acetylcholine receptor antagonist scopolamine can generate a rapid and sustained antidepressant effect (Furey and Drevets, 2006, Drevets and Furey, 2010). Moreover, the mode of action of scopolamine seems to involve AMPA receptor activation as well as the mTOR pathway, in analogy with ketamine (Voleti et al., 2013).

Needless to say, the underlying mechanisms of the action of ketamine in primates appear to be complex. Thus, for example, a recent study in monkeys reported that low doses of ketamine increased serotonergic transmission by inhibiting the activity of the SERT, which may contribute to the antidepressant effect of ketamine (Yamamoto et al., 2013).

1.11.2. Adjunctive atypical APDs in bipolar disorder and MDD

In general, most APDs are effective in ameliorating manic symptoms in bipolar disorder (Cipriani et al., 2011). Importantly, several studies show that adjunct treatment with atypical APDs can be used to augment the effect of antidepressants in bipolar depression as well as treatment-resistant MDD (Nelson and Papakostas, 2009, Cruz et al., 2010). In addition, to an enhanced antidepressant effect, in analogy with that of ketamine, combined treatment with atypical APDs and antidepressant drugs may also generate a fast onset of the antidepressant effect, which may be observed as early as within 4-5 days (Calabrese et al., 2005, Dube et al., 2007, Cruz et al., 2010, Tohen et al., 2010) as opposed to several weeks with an SSRI alone (Trivedi et al., 2006).

Thus, the atypical APD olanzapine has been found to augment the antidepressant effect of fluoxetine in treatment-resistant MDD and bipolar depression (Brown et al., 2009, Tohen et al., 2010) and a fixed combination of olanzapine and fluoxetine is marketed in the US for this purpose.

The atypical APD quetiapine has been shown to reduce depressive symptoms in MDD both as adjunct treatment as well as when used as monotherapy (Cutler et al., 2009, Bauer et al., 2010). Quetiapine exerts an antidepressant effect also in bipolar depression (Calabrese et al., 2005, McElroy et al., 2010, Young et al., 2010). The APD, quetiapine seems to induce a genuine antidepressant effect since quetiapine monotherapy reduced 9 out of 10 of the MADRS subscales, including core symptoms (Calabrese et al., 2005). One explanation may be that quetiapine produces an active metabolite in humans, norquetiapine, which differs from quetiapine in its high affinity for the NET, which has been suggested to mediate the antidepressant effect of quetiapine (Jensen et al., 2008). In contrast to the human situation, norquetiapine is however not formed in rodents to any significant extent (Hudzik et al., 2008). Quetiapine treatment has been found to induce an approximate 3:1 quetiapine: norquetiapine plasma ratio in patients (Nikisch et al., 2010) and recent PET studies data have shown that quetiapine treatment results in significant NET occupancy (Nyberg et al., 2013).

2. Specific aims of this study

- To examine whether reboxetine may provide a means, by NET inhibition, to enhance the efficacy of low doses of olanzapine, and potentially contribute to mimic some of the preclinical profile of clozapine.

- To investigate whether NET inhibition, which is generated in patients by norquetiapine, may contribute to the antipsychotic effect of quetiapine.

- To evaluate adjunctive administration of the novel atypical APD asenapine to the SSRI escitalopram on cortical monoamine output, accumbal dopamine output, cortical NMDA- and AMPA receptor-mediated transmission, respectively, as well as the effects on electrically evoked EPSPs in the mPFC.

- To analyze the neurobiological effects of adjunct administration of olanzapine when added to fluoxetine, as well as to compare these effects with the corresponding effects of a single dose of ketamine with regard to cortical NMDA and AMPA receptor-mediated transmission in the mPFC.

3. Materials and methods

3.1. Animals

Male albino rats of the Wistar strain were used for behavioral and *in vivo* microdialysis experiments and male albino Sprague Dawley rats were used for the *in vitro* electrophysiological recordings. Rats were obtained from B&K Universal, (Sollentuna, Sweden; Manuscript I and II) and Charles River (Germany; Manuscript II, III and IV). Food and water was available *ad libitum* and the rats were housed under standard laboratory conditions with controlled temperature and humidity. For behavioral experiments, rats were kept on a reversed day/night (12 h/12 h) cycle with lights off at 6 am whereas for the other experiments animals where kept on a 12 h/12 h day/night cycle with lights on at 6 am. Experiments were approved by, and conducted in accordance with, the local animal ethics committee, Stockholm North and the Karolinska Institutet.

3.2. Drugs

Olanzapine was a gift from Eli Lilly (USA), quetiapine fumarate as well as raclopride tartrate were gifts from AstraZeneca (Sweden), asenapine was a gift from Schering-Plough (UK) and Merck Sharp & Dohme Corp (MSD; UK), and escitalopram was a gift from Lundbeck A/S (Denmark). Fluoxetine was obtained from Ascent Scientific (Bristol, UK). Tetrodotoxin (TTX), bicuculline and 2-amino-3-(3-hydroxy-5-methyl-isoxazol-4-yl) propionic acid (AMPA) were obtained from Tocris (Bristol, UK). Glycine, ketamine and N-methyl-D-aspartic acid (NMDA) was obtained from Sigma (St. Louis, MO, USA). SCH23390 HCl was obtained from RBI.

3.3. Conditioned avoidance response test

The conditioned avoidance response (CAR) test is a behavioral model used to assess antipsychotic-like activity of drugs, based on Ivan Pavlov's work on conditioned stimuli (CS) and unconditioned stimuli (UCS; Courvoisier, 1956). The CAR test has been used since the 1950's and has high predictive validity for antipsychotic activity of drugs (Courvoisier, 1956, Arnt, 1982, Wadenberg and Hicks, 1999, Wadenberg et al., 2001). With slight experimental modifications, it can be used with rodents as well as with e.g. monkeys. The CAR test utilizes the propensity for APDs to reduce responding to a CS but has no or little effect on the response to an UCS, in contrast to e.g. sedative drugs, where both CS and UCS responding is impaired (Courvoisier, 1956).

The validity of the model is further supported by the fact that APDs, such as haloperidol or olanzapine, are effective in the CAR test at doses that induce similar striatal D_2 receptor occupancy to that observed in patients receiving treatment (Farde et al., 1988a, Farde et al., 1992, Wadenberg et al., 2000, Wadenberg et al., 2001). Local injections of D_2 receptor antagonists into the NAc suppress CAR responding, indicating that D_2 receptor-blockade in this is important for the effect of APDs (Wadenberg et al., 1990). The importance of increased dopamine release in the striatum for the symptoms of schizophrenia is supported by human imaging studies (c.f. introduction). However, the

CAR test is not an indirect measure of striatal D_2 receptor occupancy, since the suppression of CAR of a low, subeffective, dose of a D_2 antagonist, yielding a similar D_2 occupancy of clozapine in patients, can be potentiated by the addition of e.g. an α_2-adrenoceptor antagonist (Farde and Nordstrom, 1992, Hertel et al., 1999a, Marcus et al., 2005). In further support of this notion are the results obtained with the muscarinic agonist xanomeline, which possess antipsychotic activity (Shekhar et al., 2008) and is effective in the CAR test (Shannon et al., 2000). These data indicates that other mechanisms of APDs, in addition to D_2 receptor occupancy, contribute to the antipsychotic-like effect measured in the CAR test, as well as to the clinical effect in patients.

3.3.1. Conditioned avoidance response procedure

In the present studies, we used a two-way active avoidance test performed in conventional shuttle boxes which were divided into two compartments by a partition (Salmi et al., 1994b). Upon the presentation of a tone (the CS; 80 dB white noise), the rats had 10 s to cross the partition into the other side of the box, or the UCS (i.e. an electrical stimuli of approximately 0.3 to 0.4 mA) was delivered. The CAR system was automatic and the shuttle box was equipped with photocells connected to a computer to continuously monitor the location of the rat, controlling the CS and UCS, and record the following behavioral variables; avoidance (respond to CS within 10 s), escape (respond to CS+UCS), escape failure (failure to respond to CS and UCS within 60 s). Before the start of the experiments, rats were trained for 5 days. Once the rats learn to respond to the CS the behavior is very stable, and only rats performing > 85% avoidance were included in the study. On experimental days, experiments lasted 10 minutes and were preceded by a 10 minute pre-test. The experiments were conducted 20, 90 and 240 min (manuscript I) or 5 and 30 min (manuscript II) after injection. Before the start of each experimental session the rats were habituated to the box for 5 min. Experimental days where always separated by two non-experimental days. All rats received all treatments in a counterbalanced change-over design, and thus serving as their own controls (Li, 1964).

Data obtained in the CAR experiments are not normally distributed and accordingly, non-parametric tests were used. CAR data was analyzed using Friedman's two-way ANOVA using STATISTICA software [Statsoft Inc, USA] followed by Wilcoxon matched-pairs signed-ranks test. In all tests, $p<0.05$ was considered statistically significant.

3.4. Catalepsy

The measurement of catalepsy in animals is a model with high predictive validity of EPS liability in patients (Arnt et al., 1981, Wadenberg, 1996). In rats, catalepsy can be defined as "a drug-induced state where the animal, when placed in an awkward or unnatural position, will remain in this position for a significantly longer time than vehicle-treated control animals" (Wadenberg, 1996). APDs are thought to induce EPS and catalepsy by blockade of D_2 receptor in the dorsal striatum (Farde et al., 1992, Nordstrom et al., 1993, Wadenberg et al., 2001). The level of D_2 receptor blockade at

which a drug has high risk of catalepsy in rodents and EPS in humans is similar (Nordstrom et al., 1993, Wadenberg et al., 2000, Wadenberg et al., 2001). Consequently, drugs with high risk of EPS (e.g. haloperidol) induce catalepsy in rodents and *vice versa*, drugs with low risk of EPS in patients (e.g. clozapine and quetiapine) do not (Wadenberg et al., 2001, Kapur et al., 2002). Anticholinergic drugs are used to ameliorate antipsychotic-induced parkinsonism and can also reverse catalepsy induced by D_2 receptor antagonists (Arnt, 1982).

3.4.1. Catalepsy procedure

The catalepsy experiments in the present studies were performed in a dimly lit room. At 30, 90 and 120 min after drug administration the rats were placed on an inclining grid with an angle of 60°, and, after a 30 s adaptation period, the time to the first paw movement was measured. To minimize the risk of bias affecting the scoring, the effect of the drug or drug combinations on catalepsy was scored by an observer blind to the treatment.

The recorded time to the first paw movement (in minutes) rendered a score (from 0 to 5) according to a scale where the intervals are based on a square root transformation of the time: 0.00-0.08 min=0; 0.09-0.35 min=1; 0.36-0.80 min=2; 0.81-1.42 min=3; 1.43-2.24 min=4: 2.25 min≥5 (Ahlenius and Hillegaart, 1986). A score below 2 indicates low propensity to induce catalepsy (Wadenberg et al., 2001). Data from the catalepsy measurements are not normally distributed and therefore non-parametric statistical tests were used. Catalepsy scores were analyzed by Kruskal-Wallis one-way ANOVA followed by Mann-Whitney *U*-test using STATISTICA software. In all tests, $p<0.05$ was considered statistically significant.

3.5. In vivo microdialysis

Microdialysis is a technique that allows the continuous measurement of biologic molecules such as neurotransmitters from tissues and organs, in living and awake animals with minimal tissue damage over long time periods (hours to days; see (Ungerstedt and Pycock, 1974, Ungerstedt, 1991). A dialysis probe is implanted in the organ of interest and perfused with a perfusion solution, which is collected and can be analyzed using conventional analytical methodologies. The semipermeable dialysis membrane allows for the diffusion of small (e.g. neurotransmitters) but not large molecules (e.g. proteins). Molecules in the extracellular space equilibrate with the perfusion solution and the concentration of the molecules of interest in the perfusate correlates with the concentration in the surrounding extracellular compartment. Microdialysis is widely used in pharmacology and neuroscience to monitor the release of neurotransmitters in brain of rodents. In addition to measuring the extracellular content of biological molecules, it can also be used to administer substances (e.g. drugs or neurotransmitters) directly into the organ of interest with high spatial specificity. The samples are collected and analyzed outside the tissue, which allows for the analysis of all molecules of interest in each sample and the direct comparison with a known standard.

However, the microdialysis technique has some important limitations. It is invasive and inevitably causes tissue damage in the area around the probe. Therefore, surgical implantation must be performed several hours, often days, prior to the experiment. Moreover, due to limitations in sensitivity of the method of analysis, the dialysate must be collected for some time (up to 30 min in our experiments) to obtain sufficient mass of the substance of interest to allow analysis. As a consequence, microdialysis lacks the temporal resolution obtained with e.g. biosensors. Therefore, microdialysis is less well suited for the measurement of rapid changes in neurotransmitter release. Moreover, recovery of molecules from the extracellular space is, in addition to the extracellular concentration, dependent on the diffusion over the probe membrane and the diffusion in the extracellular space, which may differ between different probes and animals. Therefore, in the present studies, we have analyzed changes in neurotransmitter output, which then compensates for these technical differences between animals.

3.6.1. In vivo microdialysis procedure

In our experiments, rats were anesthetized, placed in a stereotactic frame and surgically implanted with a concentric dialysis probe. Dialysis probes were made in-house. The probes were implanted in the mPFC and NAc according to the atlas of Paxinos and Watson (Paxinos and Watson, 1998) and anchored to the scull with screws and dental cement. Rats were allowed 48 h recovery before the start of the experiment. Microdialysis experiments were performed in awake, freely moving, rats. During the experiments, dialysis probes were perfused with physiological perfusion solution at a constant flow rate of 2.5 µl/min and collected for 30 min (mPFC) or 15 min (NAc) for analysis.

After collection, the perfusate was automatically injected on a high performance liquid chromatography (HPLC) system. Separation of neurotransmitters and metabolites were performed by reversed phase chromatography on a C-18 separation column. Samples were analyzed using electrochemical detection in a high-sensitive analytical cell (model 5111; ESA Bioscience) controlled by a potentiostat with applied potentials of 400 mV for detection of metabolites and -200 mV for detection of dopamine, noradrenaline and serotonin. Injections of drugs were performed after the output of neurotransmitters and metabolites was stable. The correct placement of the probe was verified after the experiment in sections of the relevant brain region stained with neutral red.

Microdialysis data was analyzed using the Totalchrome software (Perkin Elmer, USA) which generates both a peak area and peak height for each analyte and sample. The obtained retention time and peak area of the sample was compared to that of a known standard and the value was expressed as fmol/min. In neither study, did the basal concentrations of the analytes differ between the groups in the respective brain area (one-way ANOVA), and the data was subsequently expressed as percent of baseline (i.e. the mean output of the two [mPFC] or four [NAc] samples preceding the drug injection).

Statistical evaluation of microdialysis data over time was performed by a repeated measures two-way (treatment x time) ANOVA. To analyze the overall effect of the different treatments we analyzed the mean neurotransmitter output in studies I and III in the interval 60-240 min for mPFC and 45 -240 min for NAc, and in study II in the intervals 60-180 min for mPFC and 45 -180 min for NAc . The between groups comparison of the overall effect was analyzed using a one-way ANOVA followed by planned comparisons of least square means. Effect of treatment was statistically evaluated using STATISTICA software. In all tests, $p<0.05$ was considered statistically significant.

3.6. In vitro electrophysiological recordings

Hodgkin and Huxley where the first to use intracellular recordings to study electrical properties of neurons (Hodgkin and Huxley, 1939). By the late 1940's Marmont and Cole developed a voltage clamp technique, which Hodgkin and Huxley utilized to study the mechanisms underlying the generation of action potentials in giant axons of squids (see e.g. (Hodgkin et al., 1952). Subsequently, in the 1970's, Neher and Sakmann developed the patch clamp technique, which made it possible to measure conductance of single ion channels (see e.g. Neher and Sakmann, 1976).

The voltage clamp technique allows the experimenter to hold (or clamp) the membrane potential of a cell at a fixed value, preventing the activation of voltage dependent ion channels which allow recordings or characterizations of activated ligand-gated ion channels. In the voltage clamp mode, the ion flow generated by the activation of a ligand-gated ion channel is counterbalanced by a current in the opposite direction, generated by a voltage-controlled current source, to keep the membrane potential steady. The measured current generated by the amplifier is proportional to the current generated by the ligand-gated ion channels.

Electrophysiological recordings of cells in brain slices *in vitro* have several advantages in the study of ion channels. In a slice, electrophysiological measurements are not disturbed by fluctuations due to blood flow and breathing of the animal and the content of the perfusion solution with regards to e.g. ion concentration as well as drug concentrations can be easily manipulated. The placement of the recording electrode is also visible to the eye.

Dopamine release has been shown to occur in rat brain slice preparations e.g. via activation of NMDA receptors. This effect was found to be partly TTX insensitive (Krebs et al., 1991). Exocytosis of neurotransmitters may occur at a synapse, even without presynaptic stimulation, which can be recorded as miniature EPSP. Previous studies from our group have shown that depletion of monoamines prevents the facilitating effect of a combination of idazoxan and raclopride on NMDA receptor-mediated synaptic transmission in the slice and that this effect was rescued by L-DOPA (Marcus et al., 2005), clearly demonstrating the importance of catecholamines in our experiments.

3.6.1. Preparation of brain slices

Rats were decapitated under halothane anesthesia and the brain was cooled in ice-cold Ringer's solution. The brain was cut coronally on a vibratome into 450 µM slices after which they kept in aerated Ringer's solution for >1 h before experiments to allow for recovery. A slice containing the mPFC was transferred to the recording chamber (30 °C) and was held submerged between two nylon nets in aerated Ringer's solution. The chamber was perfused continuously using a gravitational system with a flow-rate of 1-2 ml/min. Penetration of pyramidal cells in layer V or VI with sharp electrodes was performed blindly. Electrodes were manufactured from borosilicate glass capillaries (tip resistance of 55-140 MΩ) using a horizontal electrode puller and were filled with 2 M potassium acetate.

3.6.2. Intracellular recordings

The experiments were recorded using an Axoclamp 2A or 2B amplifier (Molecular Devices, USA) connected to a PC running Clampex 9.2 software (Molecular Devices, USA), via a digital/analogue interface. Single electrode voltage-clamp recordings were performed in the discontinuous mode (sampling rate 5-6.2 kHz) at a holding potential of -60 mV. All drugs, as well as NMDA (5-15 µM) and AMPA (2.5- 5 µM), were applied by bath perfusion. The effect of NMDA or AMPA induced currents was recorded before (control) and after 5 and 30 min of drug application.

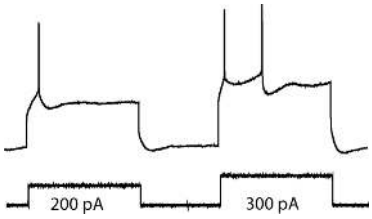

Figure 9. *Electrophysiological trace showing injection of 2 square pulses (1000 ms) of positive current (200 and 300 pA) into a presumed pyramidal cell of the rat mPFC, in response to which action potentials were elicited.*

Presumed pyramidal cells were distinguished from non-pyramidal cells using criteria published previously (Connors and Gutnick, 1990, Arvanov and Wang, 1997). Presumed pyramidal cells have relatively long spike duration (1-3 ms at half maximum spike amplitude) and show a pronounced spike-frequency adaptation in response to constant current-depolarization pulses, in contrast to non-pyramidal cells, which have relatively short spike duration (< 1 ms at half maximum spike amplitude) and generally do not show spike-frequency adaptation. In slice preparations of the PFC four types of pyramidal cells can be distinguished by their morphological and corresponding electrophysiological properties: the regular spiking, intrinsic bursting, repetitive oscillatory bursting and the intermediate type (Yang et al., 1996).

Excitatory postsynaptic potentials (EPSPs) are transient depolarizations of the cell membrane due to the influx of cations via the activation of ligand-gated ion channels. In pyramidal cells the EPSP consist of both an early, AMPA-mediated phase, and a prolonged, late NMDA-mediated phase (Tanaka and North, 1993, Chen and Yang, 2002). The opposite, an inhibitory postsynaptic potential (IPSP) is caused by an influx of negative ions or outflux of positively charged ions from the cell. IPSPs in pyramidal

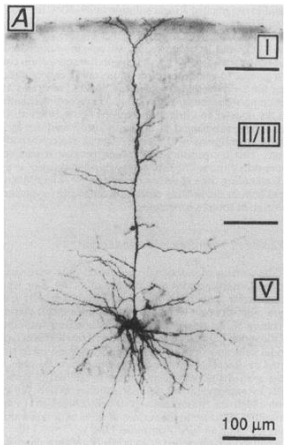

Figure 10. *Micrograph showing a layer V rat pyramidal cell injected with biocyntin (Kawaguci 1993). Cortical layers are denoted by roman numerals. Layer V-VI pyramidal cells have a triangular (or pyramidal) shaped soma with a size averaging approximately 20 µM.*

cells of the mPFC are blocked by the $GABA_A$ antagonist bicuculline (Chen and Yang, 2002).The effect of several EPSPs are additive and if the cell membrane is sufficiently depolarized over a threshold value, voltage gated ion channels are activated and an action potential is elicited. The atypical APD clozapine has been found to induce voltage dependent sodium channel-dependent spikes overriding the EPSP. These spikes have variable onset latencies and generated by a polysynaptic input to the layer V pyramidal cell. However, the action potentials are prevented by NMDA receptor antagonists and are thus dependent on NMDA receptor activation (Chen and Yang, 2002).

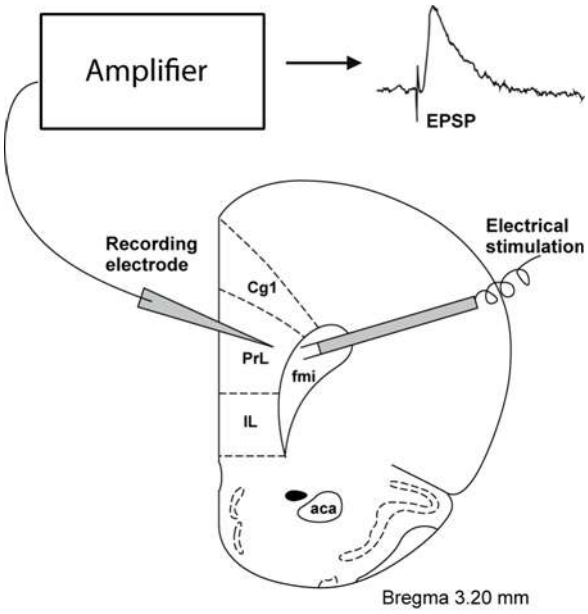

Figure 11. *Cartoon illustrating the set-up for eliciting and recording EPSPs in pyramidal cells of the rat mPFC. Shown are the positions of the stimulation electrode and the recording electrode. For voltage clamp experiments the set-up is similar, but without the stimulation electrode. Cg1- Cingulate cortex area 1; PrL-Prelimbic cortex; IL-Infralimbic cortex; fmi-forceps minor; aca-anterior commissure. Modified from* (Paxinos and Watson, 1998).

In the present study, electrically evoked EPSPs where achieved by placing two stainless steel electrodes in the forceps minor (white matter) proximal to the mPFC and close to the recording electrode, in similarity to previously published experiments (figure 11) (Arvanov et al., 1997, Chen and Yang, 2002, Jardemark et al., 2012). To elicit EPSPs, trains of three square pulses of 0.3 ms (11 to 31 mV) at a rate of 0.05 Hz were passed between the tips and the evoked change in membrane potential (i.e. the EPSP) was recorded in the current clamp mode in layer V pyramidal cells. The recording electrode was filled with 2 M potassium acetate and bicucculine (2µM) was routinely included in

the perfusion solution to inhibit $GABA_A$ mediated responses. To evaluate the effect of drug treatment, a stimulation potential eliciting a sub-maximal response (i.e. EPSP) was chosen and the effect was recorded before and after 5, 15, 15 and 35 minutes of drug treatment. The effect of drugs or drug-combination was evaluated both qualitatively, for their ability to facilitate the induction of action potentials, as well as their effect on the total area of the evoked EPSP.

To investigate whether ketamine pretreatment facilitates NMDA and AMPA receptor-mediated currents in our slice preparation we injected rats with ketamine (10 mg/kg i.p.) or saline (2 ml/kg) 24 h prior to the electrophysiological experiment. Preparation of brain slices and recordings of NMDA- (5 μM) and AMPA- (5 μM) induced currents were performed as previously described.

The effect of a drug or drug combination was calculated by dividing the amplitude of the AMPA- or NMDA-induced current (in pA) after drug application with the amplitude of the control AMPA- or NMDA-induced current. Paired *t*-test was used to evaluate the effect of drug treatment on NMDA- and AMPA-induced currents. Unpaired *t*-test was used to evaluate the effect of ketamine pretreatment on NMDA- and AMPA-induced currents. For multiple comparisons, one-way ANOVA followed by Tukey HSD (manuscript I) or the Newman-Keuls multiple comparison test (manuscript II, III and IV) were used. The areas of the electrically evoked EPSPs were quantified using Clampfit 9.2. Due to the large variation of the EPSP area (expressed as mV*ms) the data was first log transformed before it was analyzed using a repeated measures two-way ANOVA followed Fisher's Least Significant Difference test. The effect of treatment on AMPA- and NMDA-induced currents was statistically evaluated using STATISTICA (manuscript I) or Prism (Graphpad Prism Inc., USA; manuscript II, III and IV). EPSP data was statistically evaluated using STATISTICA. In all tests, $p<0.05$ was considered statistically significant.

4. Results and discussion

4.1. Role of concomitant NET-inhibition for the clinical effects of antipsychotic drugs

The prototypical atypical APD clozapine has been found to possess superior efficacy in treatment resistant schizophrenia compared to other APDs, even though, or maybe just because, clozapine-treatment induces a low striatal D_2 receptor occupancy. Clozapine has high affinity for the α_2-adrenoceptor, which has been suggested to be important for its superior efficacy in schizophrenia and allow for its low D_2 receptor occupancy. However, clozapine treatment is associated with severe side effects, most notably agranulocytosis, which limits its use. The atypical APD olanzapine has a structure and receptor-biding profile similar to that of clozapine, e.g. higher affinity for several serotonergic receptors compared to the D_2 receptor, but lacks affinity for the α_2-adrenoceptor. Olanzapine-treatment induces a higher D_2 receptor occupancy than clozapine and may be associated with side-effects, such as weight gain and EPS, but not with agranulocytosis. Interestingly, the antipsychotic-like effect of olanzapine was potentiated by addition of the α_2-adrenoceptor antagonist idazoxan (Wadenberg et al., 2007). In similarity to the effects of idazoxan, the NET inhibitor reboxetine has been found to enhance dopamine output in the mPFC and to facilitate the antipsychotic-like effect of raclopride (Hertel et al., 1999a, Hertel et al., 1999b, Linner et al., 2002), indicating that reboxetine may potentially also be used to augment the effect of olanzapine.

In fact, NET inhibition may contribute to the clinical effect of the atypical APD quetiapine. Quetiapine exerts its antipsychotic effect in similarity with clozapine, at an unusually low D_2 receptor occupancy and interestingly, quetiapine treatment has been found to generate an active metabolite, norquetiapine, which has high affinity for the NET. Norquetiapine has previously been suggested to mediate the antidepressant effect of quetiapine (Jensen et al., 2008). However, its contribution to the antipsychotic effect of quetiapine has not been investigated. Norquetiapine is not formed in rodents to any major extent (Hudzik et al., 2008), making rats a suitable model to study the contribution of NET inhibition to the effect of quetiapine.

4.1.1. Manuscript I

In the present study, we investigated whether concomitant NET-inhibition potentiates the efficacy of the SGA olanzapine and potentially mimic some of the preclinical effects of clozapine. We used the CAR test to investigate the effect of concomitant NET-inhibition on the antipsychotic-like activity of olanzapine and the catalepsy test to assess its effect on EPS liability. The effect on dopamine output in the mPFC and NAc were assessed using *in vivo* microdialysis in freely moving rats. Moreover, the effects of NET-inhibition combined with olanzapine on cortical NMDA-induced currents using intracellular recordings *in vitro* were also investigated.

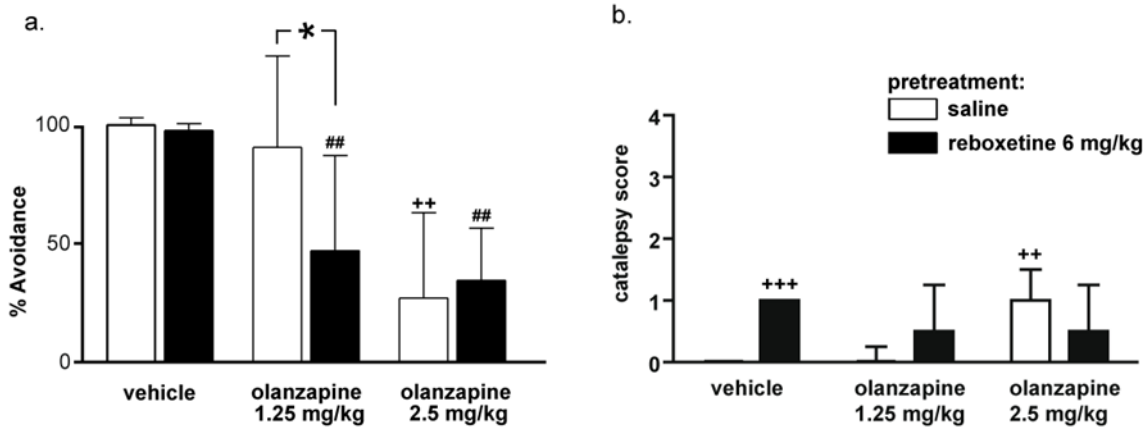

Figure 12. *Concomitant NET-inhibition by reboxetine significantly potentiates the antipsychotic-like effect of a sub-optimal (1.25 mg/kg), but not optimal (2.5 mg/kg) dose of olanzapine at 20 min after treatment without increasing the EPS liability. (a) The effect on CAR behavior at 20 min after administration of vehicle, olanzapine 1.25 or 2.5 mg/kg (i.p.) combined with saline or reboxetine (6 mg/kg i.p.). The results are presented as the median avoidance ± semi-interquartile range (%).* $^{++}$ *p<0.01 vs. saline+ vehicle,* $^{\#\#}$ *p<0.01 vs. reboxetine+ vehicle, * p<0.05 saline + olanzapine vs. reboxetine+olanzapine. (b) All treatments showed very low propensity to induce catalepsy. The catalepsy score (60 min after dose) is presented as median score ± semi-interquartile range. ++ p< 0.01, +++ p< 0.001 vs. saline+ vehicle.*

Addition of reboxetine (6 mg/kg) to olanzapine potentiated the antipsychotic-like effect (i.e. suppression of CAR) of a sub-effective (1.25 mg/kg) but not optimal (2.5 mg/kg) dose of olanzapine (figure 12a). Reboxetine (6 mg/kg) as well as olanzapine (2.5 mg/kg) significantly increased in the catalepsy score (figure 12b). However, the median scores were low, below 2 for all treatments, indicating low propensity to induce catalepsy.

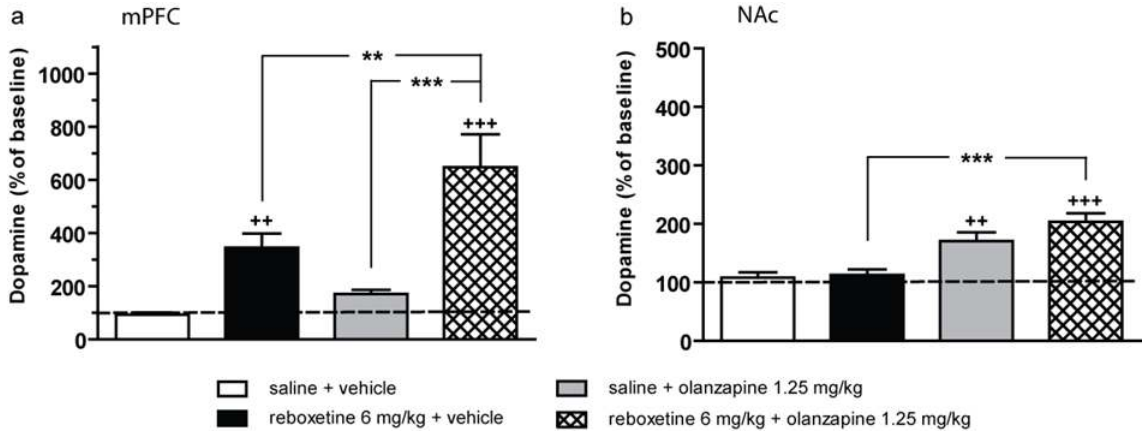

Figure 13. *Concomitant NET-inhibition by reboxetine enhances the olanzapine-induced dopamine output in the mPFC but not in the NAc. The mean dopamine output in mPFC (a) and NAc (b) of vehicle or olanzapine (1.25 mg/kg i.p.) in rats pretreated with saline or reboxetine (6 mg/kg i.p.). The results are presented as mean ± SEM.* $^{++}$ *p<0.01,* $^{+++}$ *p< 0.001 vs. control group (saline/vehicle); **p<0.01, *** p<0.001 indicate between treatment effects.*

The enhanced suppression of CAR obtained when reboxetine was added to olanzapine was accompanied by a preferential increase in prefrontal dopamine output, without affecting the olanzapine-induced dopamine output in the NAc (figure 13 a, b).

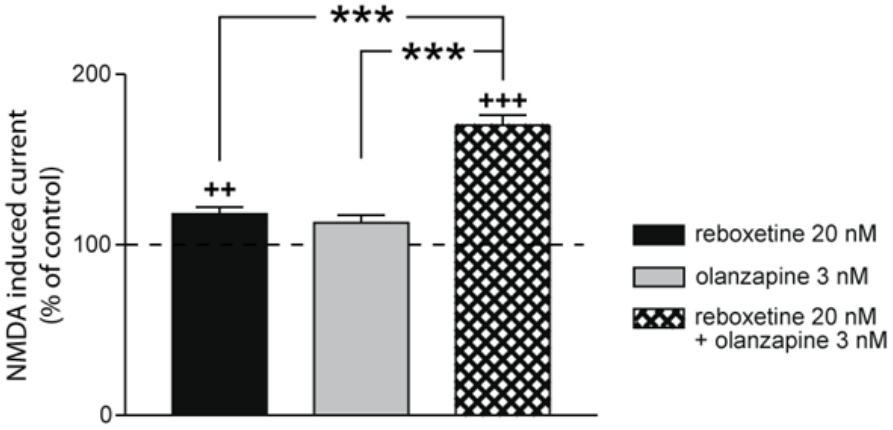

Figure 14. *Addition of reboxetine to a sub-effective concentration of olanzapine significantly enhances the NMDA-induced currents in pyramidal cells of the rat mPFC. Reboxetine (20 nM) produced a small but significant increase in the NMDA-induced currents. Addition of reboxetine (20 nM) to olanzapine (3 nM) significantly increased these currents compared to each drug given alone.* $^{++}$ *p<0.01,* $^{+++}$ *p<0.001 vs. baseline. *** p< 0.001 between different treatments. The results are presented as mean ± SEM. The holding potential was -60 mV.*

Addition of reboxetine to a sub-effective concentration of olanzapine enhanced NMDA-induced currents in pyramidal cells from the rat mPFC (figure 14).

4.1.2. Manuscript II

In the present study, we used reboxetine as a model compound due to its high specificity for the NET, to investigate *in principle*, whether NET-inhibition, obtained in patients by the active metabolite norquetiapine, contributes to the antipsychotic effect of quetiapine.

The effect of concomitant NET-inhibition on the antipsychotic-like effect of quetiapine was studied using the CAR model. The effect on dopamine and DOPAC output was assessed using microdialysis and the effect on NMDA-induced currents was studied using *in vitro* intracellular recordings. In addition, we investigated the effects of adding reboxetine to the selective $D_{2/3}$ receptor antagonist raclopride on cortical NMDA-induced currents.

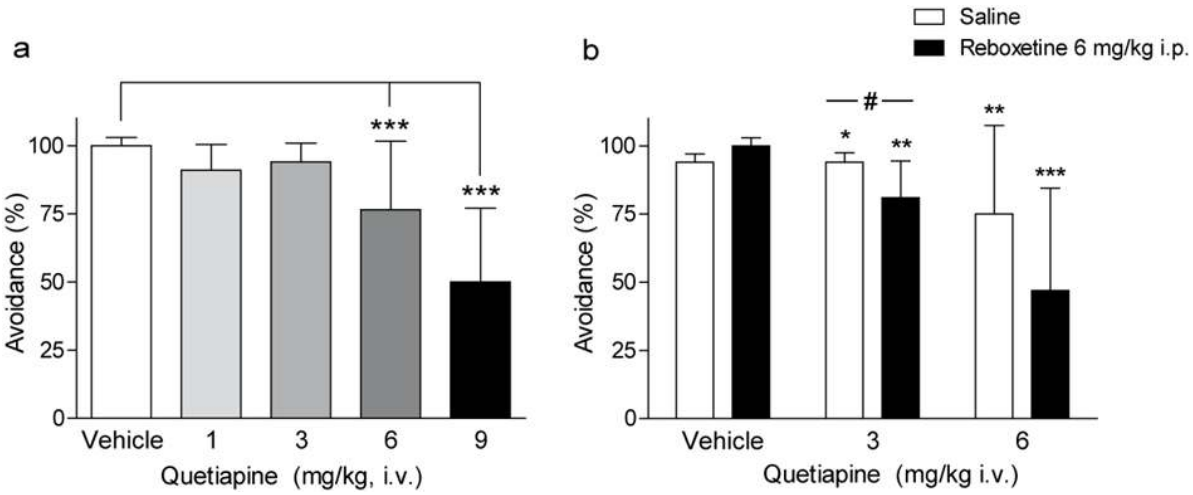

Figure 15. *Addition of reboxetine to quetiapine potentiates the quetiapine-induced suppression of conditioned avoidance behavior. (a) The effect of quetiapine (1, 3, 6 and 9 mg/kg i.v.; n=12) on CAR behavior. (b) The effect of quetiapine alone and after pretreatment with reboxetine (6 mg/kg; n=11) . The results are presented as median avoidance (%) ± semi-interquartile range. *p<0.05, **p<0.01, *** p<0.001 vs. vehicle, # p< 0.05 as indicated in the figure.*

Quetiapine, given i.v., produced a short-lasting suppression of the CAR behavior at 6 and 9 mg/kg (figure 15a). Pretreatment with reboxetine (6 mg/kg i.p.) produced a small but significant potentiation of the antipsychotic-like effect of quetiapine at 3 mg/kg (figure 15b). Reboxetine pretreatment seemed to facilitate the suppression of CAR behavior also of the higher dose of quetiapine (6 mg/kg) but this effect did not reach statistical significance.

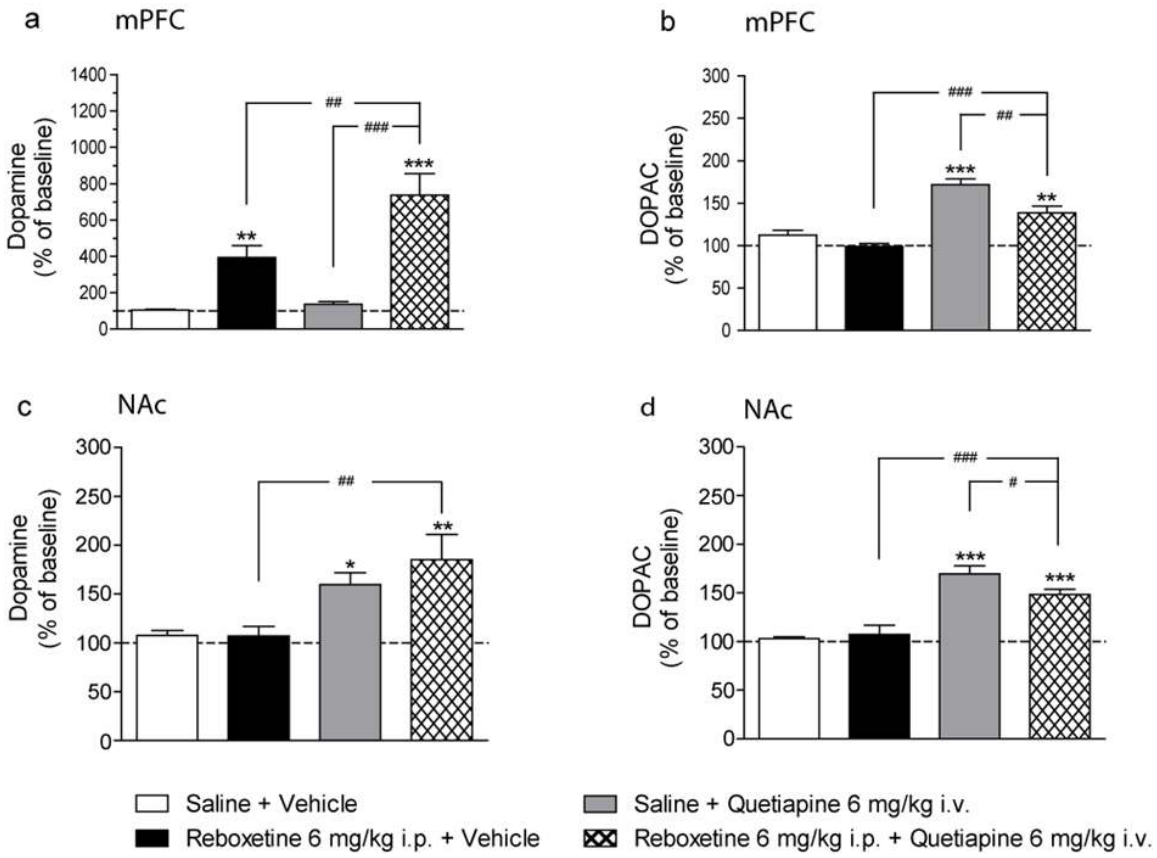

Figure 16. *Addition of reboxetine to quetiapine enhances the dopamine output in the mPFC but not in the NAc. Effects of quetiapine (6mg/kg i.v.) and reboxetine (6 mg/kg i.p.) on dopamine (a, b) and DOPAC (c, d) output in the mPFC and NAc respectively. The results are presented as mean ± SEM. **p< 0.01, ***p<0.001 vs. control group (i.e. saline+vehicle).* $^{\#}p<0.05$, $^{\#\#}p<0.01$, $^{\#\#\#}p<0.001$ *comparisons as indicated in the figure.*

Addition of reboxetine to quetiapine induced a large increase in the dopamine output in the mPFC but not in the NAc (figure 16 a, c). The increased cortical dopamine output was accompanied by a reduction in the DOPAC output (16 b).

Quetiapine facilitated NMDA-induced currents (figure 17b), in similarity to previously published results (Ninan et al., 2003b). Addition of reboxetine to a sub-effective concentration of quetiapine enhanced the NMDA-induced currents compared to each drug when given alone (figure 17c). The facilitatory effect was prevented by the addition of the dopamine D_1 receptor antagonist SCH23390. Addition of reboxetine to raclopride also increased the NMDA-induced currents compared to each drug given alone (figure 17d).

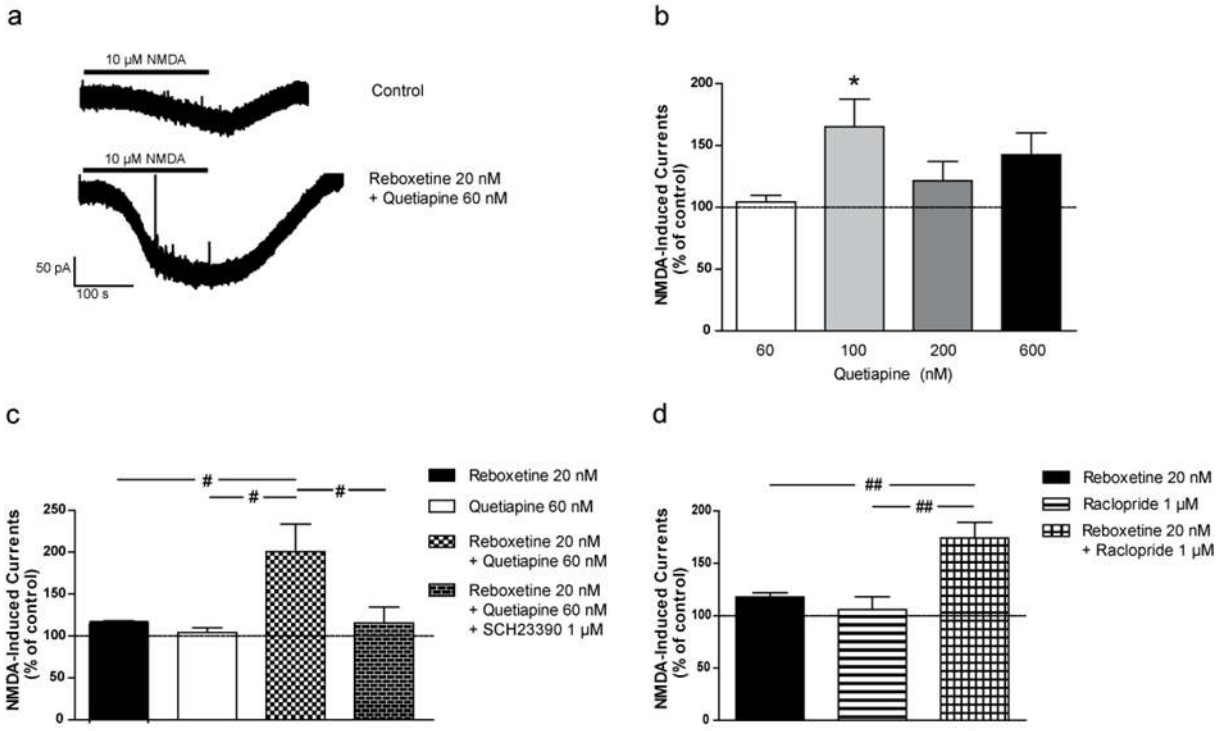

Figure 17. *Concomitant NET-inhibition potentiates the effect of quetiapine and raclopride on NMDA-induced responses compared to each drug given alone in pyramidal cells of the rat mPFC. (a) Representative electrophysiological traces illustrating the effect of combined quetiapine and reboxetine on the NMDA-induced currents in pyramidal cells of the mPFC. (b) concentration-response curve of the effect of quetiapine on NMDA-induced currents. (c) The effect on NMDA-induced currents of a sub-effective concentration of quetiapine (60 nM), reboxetine (20 nM), the combination of quetiapine and reboxetine, and the effect of quetiapine and reboxetine in the presence of the dopamine D_1 receptor antagonist SCH23390. (d) The effect of reboxetine (20 nM), the $D_{2/3}$ receptor antagonist raclopride (1 µM) and the combination of reboxetine (20 nM) and raclopride (1µM) on NMDA-induced currents. The results are presented as mean ± SEM. *p<0.05 compared to baseline. #p<0.05, ##p<0.01, between groups comparison as indicated in the figure.*

4.1.3. Discussion: Role of concomitant NET-inhibition for the clinical effects of antipsychotic drugs

Addition of reboxetine potentiated the antipsychotic-like effect of a sub-effective dose of olanzapine, without inducing catalepsy, indicating that adjunctive treatment with reboxetine may allow for a dose reduction of olanzapine with maintained antipsychotic effect. In similarity, addition of reboxetine also potentiated the antipsychotic-like effect of quetiapine, which suggests, *in principle*, that NET-inhibition provided in patients by the metabolite norquetiapine, contributes to the antipsychotic effect of quetiapine, which is obtained despite its relatively low D_2 receptor occupancy. These results are in similarity to previous studies from our group investigating addition of a NET-inhibitor and an α_2-adrenoceptor antagonist to low doses of a $D_{2/3}$ receptor antagonist (Hertel et al., 1999a, Linner et al., 2002).

Addition of reboxetine to both olanzapine and quetiapine preferentially enhanced the dopamine output in the mPFC, without affecting the dopamine output in the NAc. In parallel, the DOPAC output in the mPFC was decreased when reboxetine was added to

quetiapine. A reduction of the intracellularly derived metabolite DOPAC in the mPFC when reboxetine is added to quetiapine indicates that the enhanced dopamine output may stem from an enhanced dopamine turnover generated by an increased VTA cell firing induced by the APD (Gessa et al., 2000, Yamamura et al., 2009). The enhanced turnover is not observed as increased dopamine output when the APD is given alone, as the released dopamine can be cleared from the extracellular space by the NET. Reboxetine, by blocking the NET, thus unmasks the enhanced turnover. Another contributing mechanism may be blockade of D_2 autoreceptors by olanzapine and quetiapine, disinhibiting dopamine outflow (Westerink et al., 2001).

Concomitant NET-inhibition facilitated the NMDA-induced currents of olanzapine, quetiapine and raclopride compared to either drug given alone. The effect of the reboxetine/quetiapine combination was mediated via D_1 receptor activation similar to what has been demonstrated in previous studies (Chen and Yang, 2002, Ninan and Wang, 2003, Marcus et al., 2005). Given the crucial importance of D_1 and NMDA receptor mediated transmission for cognitive function and the observed cognitive deficits in schizophrenia these results, indicate that APD-treatment with concomitant NET-inhibition, may, by facilitation of dopaminergic and NMDA receptor-mediated transmission, serve to ameliorate cognitive deficits as well as both depressive and negative symptoms in schizophrenia, and may thus be an underlying mechanism contributing to the pro-cognitive effect of quetiapine treatment obtained in schizophrenia (c.f. 1.8.2).

The increased cortical dopamine output may also contribute to the enhanced antipsychotic-like effect *per se*, since dopamine acting on D_1 receptors in the mPFC has been found to suppress subcortically derived D_2 receptor-mediated behaviors (c.f. 1.8.2).

Previous clinical studies investigating adjunctive treatment with reboxetine to APD treatment in schizophrenia yielded both positive and negative results (Schutz and Berk, 2001, Raedler et al., 2004). However, present data indicates that one of the potential benefits of concomitant NET-inhibition would be obtained at reduced dosage of APD rather than at standard doses of APDs. A dose-reduction of olanzapine enabled by addition of reboxetine, with ensuing reduced D_2 receptor occupancy, or norquetiapine in quetiapine-treated patients, may not only reduce the risk of side-effects (e.g. EPS) (Kapur et al., 2000), but also reduce the risk of drug-induced negative symptoms, cognitive deficits, negative mood and impaired reward prediction associated with high D_2 receptor occupancy (Carpenter, 1996, Saeedi et al., 2006, Kirsch et al., 2007).

Moreover, previous studies have suggested that enhanced cortical catecholamine output may underlie the beneficial effects of addition of atypical APDs to SSRIs in treatment-resistant MDD and bipolar depression (c.f. 1.11.2). Therefore, the marked facilitation of prefrontal dopamine output observed in the present studies thus proposes that NET-inhibition, in combination with the properties of an atypical APD, may contribute to relieve depressive symptoms.

4.2. Effects of low doses of atypical antipsychotic drugs added to SSRIs on monoaminergic and glutamatergic neurotransmission in the mPFC.

Addition of low to moderate doses of atypical APDs has been found to potentiate the antidepressant effect of antidepressants in both bipolar depression as well as in treatment-resistant MDD, with a rapid onset of the effect (see e.g. Dube et al., 2007, Nelson and Papakostas, 2009). Previous preclinical studies, investigating addition of the atypical APD olanzapine to the SSRI fluoxetine, have suggested that this effect may, at least partly, be due to the increased catecholamine output in the mPFC (Zhang et al., 2000). Moreover, addition of low, sub-effective concentrations of APDs to SSRIs has also been found to facilitate NMDA receptor-mediated transmission in pyramidal cells of the rat mPFC (Marcus et al., 2012). Preclinical studies investigating the mechanism of action of ketamine and scopolamine, show that the antidepressant-like effect is critically dependent on activation of AMPA receptors, and subsequently, on intracellular mechanisms involving the mammalian target of rapamycin (mTOR) pathway in the mPFC (Maeng et al., 2008, Li et al., 2010, Voleti et al., 2013, but see also Autry et al., 2011). Ketamine and scopolamine treatment was found to induce synapse formation and to increase e.g. the number of AMPA receptor GluR1 subunits in the synapses and to increase glutamatergic transmission in pyramidal cells of the rat mPFC (Li et al., 2010).

4.2.1. Manuscript III

Asenapine is a novel APD used in bipolar disorder and in the present study we investigated the potential utility of asenapine as an adjunct to the SSRI escitalopram. The effects of add-on of low doses of asenapine to escitalopram on dopamine, noradrenaline and serotonin output in the mPFC and dopamine output in the NAc were investigated using *in vivo* microdialysis in freely moving rats. Furthermore, we investigated the effects of the drug combination on NMDA and AMPA receptor-mediated currents as well as the effect on electrically evoked excitatory post-synaptic potentials (EPSPs) using intracellular recordings of pyramidal cells *in vitro*.

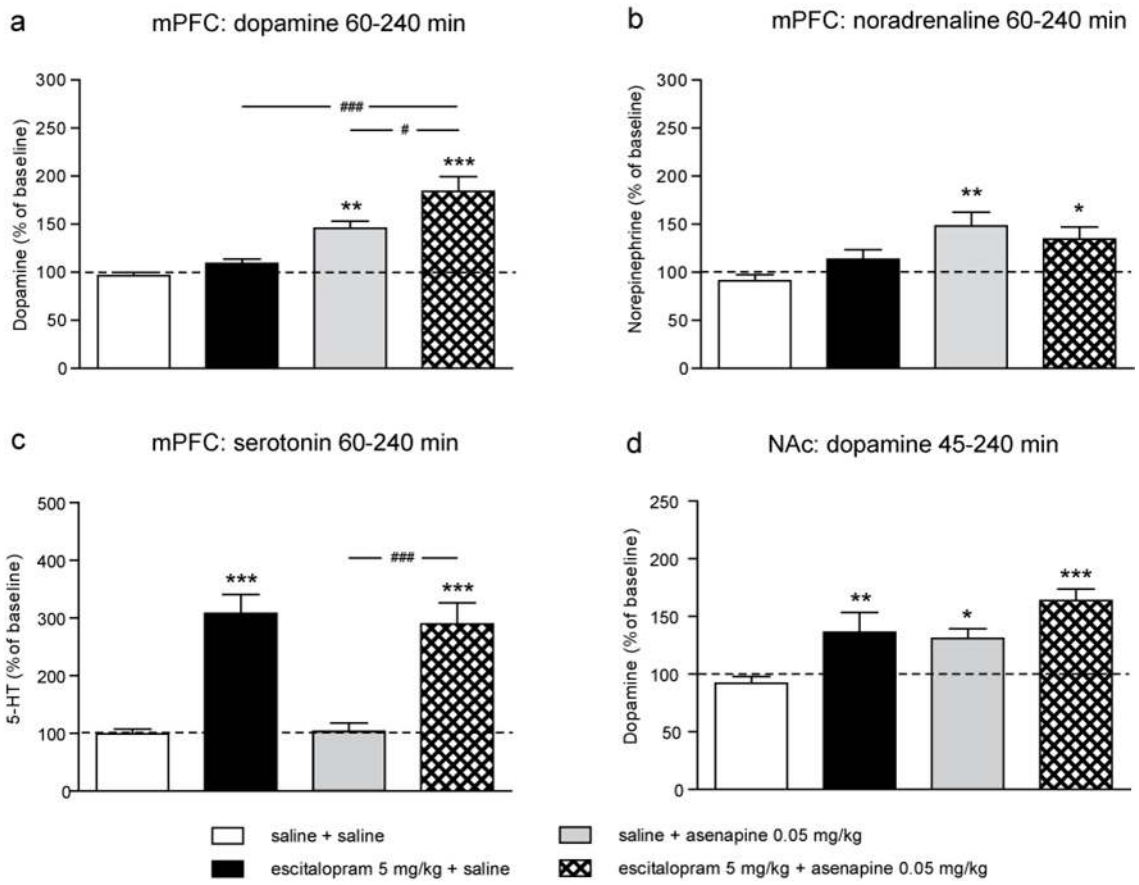

Figure 18. *Addition of asenapine to escitalopram enhances dopamine output in the mPFC. Effects of escitalopram (5 mg/kg s.c.), asenapine (0.05 mg/kg s.c.), given alone and in combination on the mean output of dopamine (a), noradrenaline (b) and serotonin (c) in the mPFC and mean dopamine output in the NAc (d). The dotted line represents baseline (100 %). The results are presented as mean ± SEM. *p<0.05, **p<0.01, ***p<0.001 vs. control (i.e. saline + saline). $^{\#}$p<0.05, $^{\#\#\#}$p<0.001 between groups comparison as indicated in the figure.*

Asenapine (0.05 mg/kg) increased the dopamine output in the mPFC, an effect that was further enhanced when asenapine was combined with escitalopram (5 mg/kg; figure 18a). Both escitalopram and asenapine increased dopamine output in the NAc, but there was no further increase when the two drugs were combined (figure 18d). Asenapine (0.05 mg/kg) increased the noradrenaline output (figure 18b) and escitalopram increased the serotonin output (figure 18c) in the mPFC. However, the output of these monoamines was not further increased when the two drugs were combined.

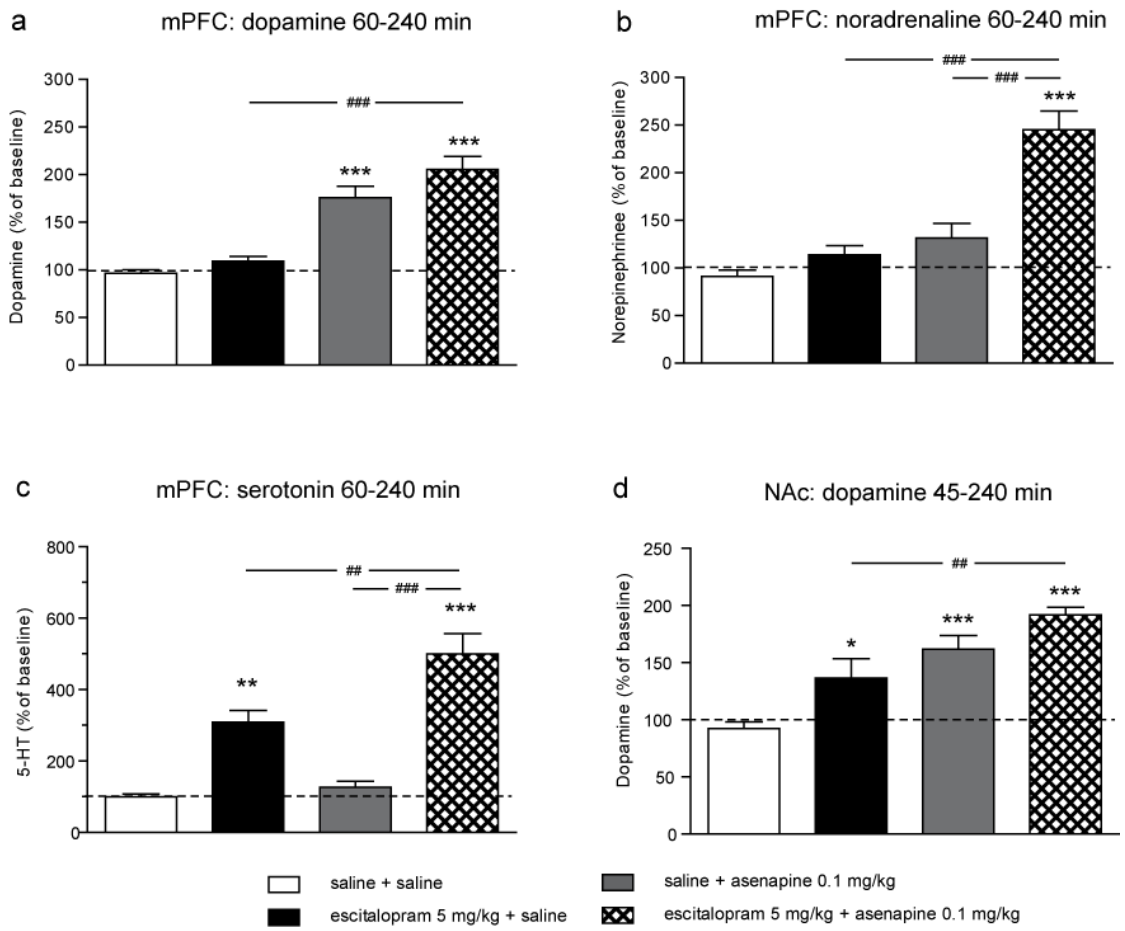

Figure 19. *Addition of a higher dose of asenapine to escitalopram increases noradrenaline and serotonin output in the mPFC. Effects of escitalopram (5 mg/kg s.c.), asenapine (0.1 mg/kg s.c.), given alone and in combination on the mean output of dopamine (a), noradrenaline (b) and serotonin (c) in the mPFC and mean dopamine output in the NAc (d). The dotted line represents baseline (100 %) The results are presented as mean ±SEM. *p<0.05, **p<0.01, ***p<0.001 vs. control (i.e. saline + saline). ##p<0.01, ###p<0.001 between groups comparison as indicated in the figure.*

A higher dose of asenapine (0.1 mg/kg) enhanced the dopamine output in the mPFC but in contrast to the effect of the lower dose of asenapine (0.05 mg/kg), the effect was not further increased when combined with escitalopram (figure 19a). Asenapine also increased the dopamine output in the NAc but in similarity to the effect of the lower dose of asenapine in the NAc, this effect was not affected by concomitant escitalopram treatment (figure 19d). Asenapine (0.1 mg/kg) did not increase noradrenaline or serotonin output when given alone, however when combined with escitalopram the combination induced a large increase in noradrenaline (figure 19b) as well as serotonin output (figure 19c).

The combination of low, sub-effective, concentrations of asenapine (1 nM) and escitalopram (3 nM) significantly enhanced NMDA-induced currents in pyramidal cells of the rat mPFC via activation of the dopamine D_1 receptor (figure 20f) in similarity results obtained when asenapine and escitalopram was investigated separately (Jardemark et al., 2010, Schilström et al., 2011).

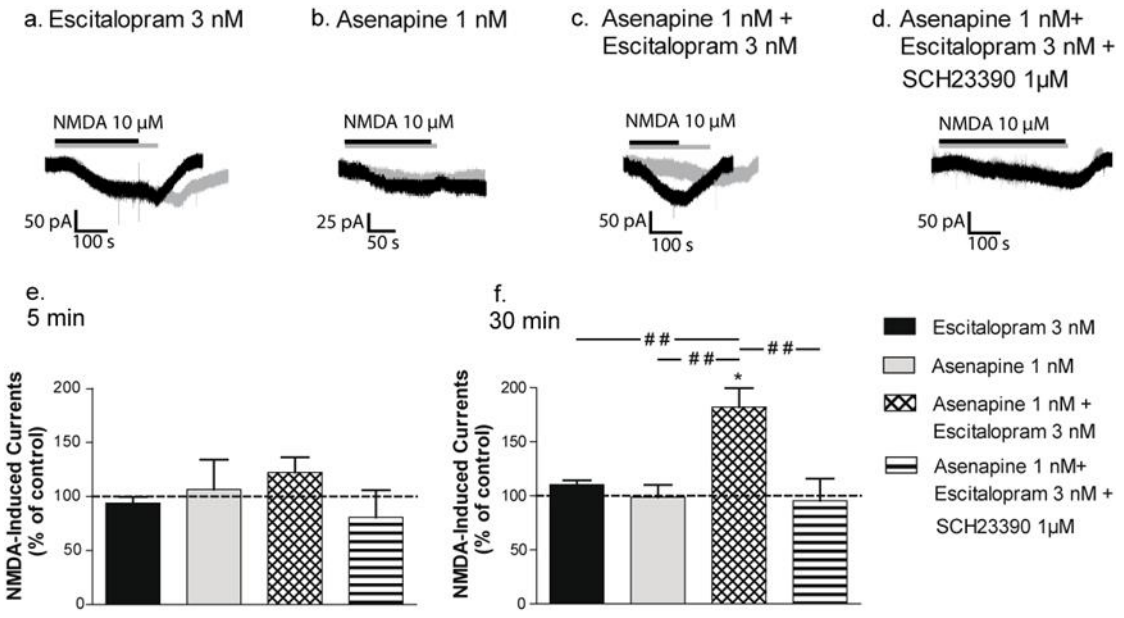

Figure 20. *A combination of asenapine and escitalopram facilitates NMDA-induced currents via activation of the dopamine D_1 receptor. Representative electrophysiological traces showing the effect of NMDA application before (grey trace) and after (black trace) application of (a) escitalopram 3 nM (b) asenapine 1 nM (c) asenapine+ escitalopram (d) asenapine+ escitalopram + SCH23390 (1 µM). The grey and black horizontal bars indicate the time of NMDA application for control and test trace, respectively. Data is summarized in bar charts 5 min (e) and 30 min (f) after drug application. The results are presented as mean ±SEM. *p<0.05 vs. control response, $^{\#\#}p<0.01$ between groups comparison as indicated in the figure.*

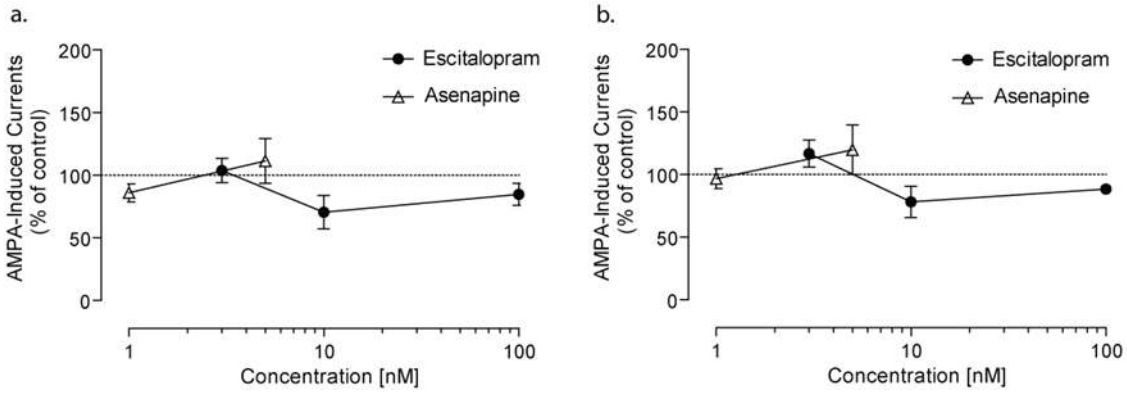

Figure 21. *Concentration-response curves for asenapine and escitalopram of AMPA-induced currents at 5 min (a) and 30 min (b) after drug application. Data are presented as mean ± SEM. The holding potential was -60 mV.*

A combination of asenapine (1 nM) and escitalopram (3 nM) also facilitated AMPA-induced currents (figure 22 e, f), an effect that was not attainable by either drug when administered alone, even at higher concentrations (21 a, b). The facilitation of AMPA-induced currents was antagonized by SCH23390 (1µM). Moreover, the combination of asenapine (1 nM) and escitalopram (3 nM) induced action potentials in all four cells tested and increased the total area of the electrically evoked EPSPs (figure 23 a to d).

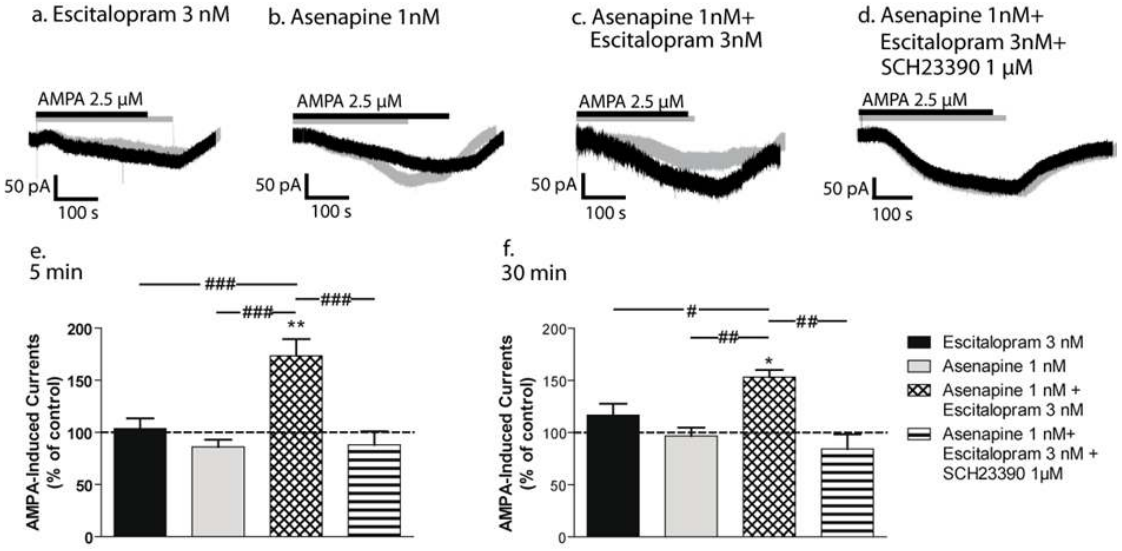

Figure 22. *A combination of asenapine and escitalopram facilitates AMPA-induced currents via activation of the dopamine D_1 receptor. Representative electrophysiological traces showing the effect of AMPA application before (grey trace) and after (black trace) application of (a) escitalopram 3 nM (b) asenapine 1 nM (c) asenapine+ escitalopram (d) asenapine+ escitalopram + SCH23390 (1 µM). The grey and black horizontal bars indicate the time of AMPA application for control and test trace, respectively. Data is summarized in bar charts 5 min (e) and 30 min (f) after drug application. The results are presented as mean ±SEM. *p<0.05, **p<0.01 vs. control response, $^{\#}p<0.05$, $^{\#\#}p<0.01$, $^{\#\#\#}p<0.001$ between groups comparison as indicated in the figure.*

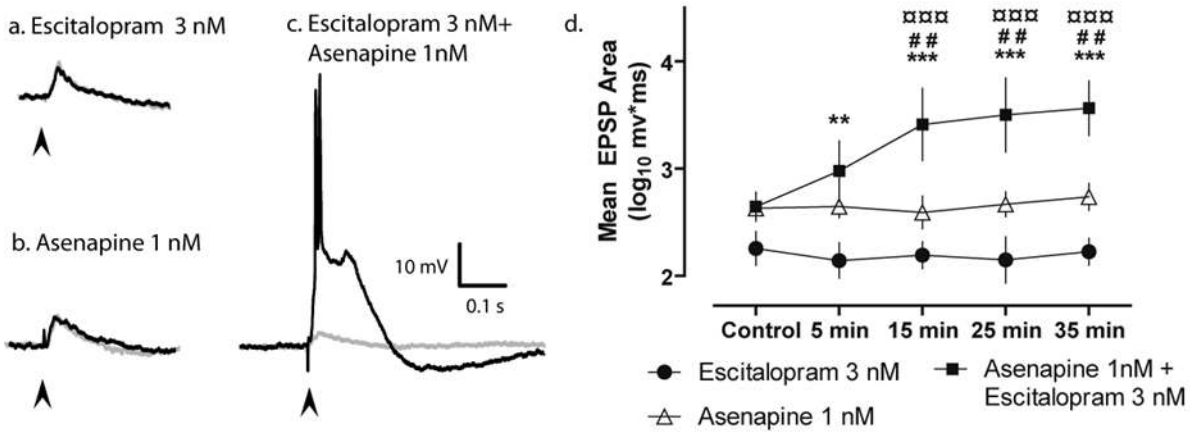

Figure 23. *A combination of asenapine and escitalopram induces action potentials and increases the area of the electrically evoked EPSPs in pyramidal cells of the rat mPFC. Representative electrophysiological traces showing the electrically evoked EPSPs before (grey) and after (black) treatment with (a) escitalopram 3 nM (b) asenapine 1 nM and (c) escitalopram 3 nM+ asenapine 1 nM. Arrows indicate time of stimulation. The logarithm of the mean EPSP area (log_{10} mV*ms) is summarized in (d). Asenapine+ escitalopram enhanced the EPSP area compared to both escitalopram (**p<0.01, ***p<0.001), asenapine ($^{\#\#}$p<0.01) as well as its own control EPSP area (i.e. the EPSP area before drug application; $^{¤¤¤}$p<0.001). The results are presented as mean ± SEM.*

4.3.2. Manuscript IV

Given the similarities in the clinical outcome between the olanzapine and fluoxetine combination and ketamine treatment (i.e. potent antidepressant action and relatively rapid onset of the effect) we investigated the effect of combined olanzapine and fluoxetine on NMDA and AMPA receptor- mediated transmission using intracellular recordings in *in vitro* slice preparations. The combination of olanzapine and fluoxetine has previously been found to increase dopamine output in the mPFC, and therefore we also investigated whether an effect on this drug combination on NMDA and AMPA receptor-mediated transmission was dependent on D_1 receptor activation. Moreover, to allow for a comparison with ketamine, the effect of a single injection of ketamine on the NMDA- and AMPA–induced currents in pyramidal cells was investigated in prefrontal brain slices 24 hours after the time of ketamine injection, by using intracellular recordings.

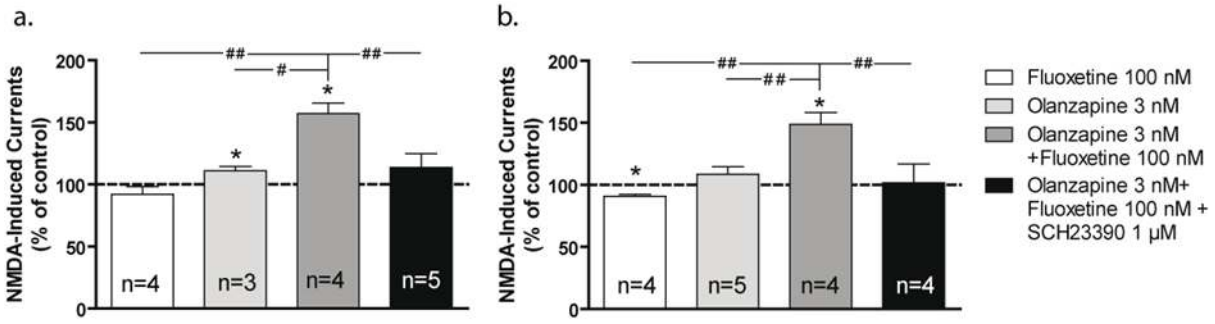

Figure 24. *A combination of olanzapine (3 nM) and fluoxetine (100 nM) facilitates NMDA-induced currents in pyramidal cells of the rat mPFC via activation of the dopamine D_1 receptor. Bar charts show the effect on NMDA-induced currents of fluoxetine (100 nM), olanzapine (3nM), fluoxetine (100 nM) + olanzapine (3 nM) and fluoxetine (100 nM) + olanzapine (3nM)+ SCH23390 (1µM) at (a) 5 min and (b) 30 min of drug administration. Data are presented as mean ± SEM (%). *p< 0.05 compared to control response. $^{\#}p<0.05$, $^{\#\#}p<0.01$ indicates a between groups effect, as indicated in the figure. The number in each bar shows group size.*

The combination of olanzapine and fluoxetine potentiated NMDA-induced currents in pyramidal cells of the rat mPFC (figure 24), an effect that was mediated via D_1 receptor activation as it was blocked by SCH23390. Interestingly, the combination of olanzapine and fluoxetine facilitated AMPA receptor-mediated currents (figure 25) even though neither drug had any effect when given alone. The facilitation of AMPA-induced currents was prevented by pretreatment with a D_1 receptor antagonist, indicating that D_1 activation was necessary for this effect.

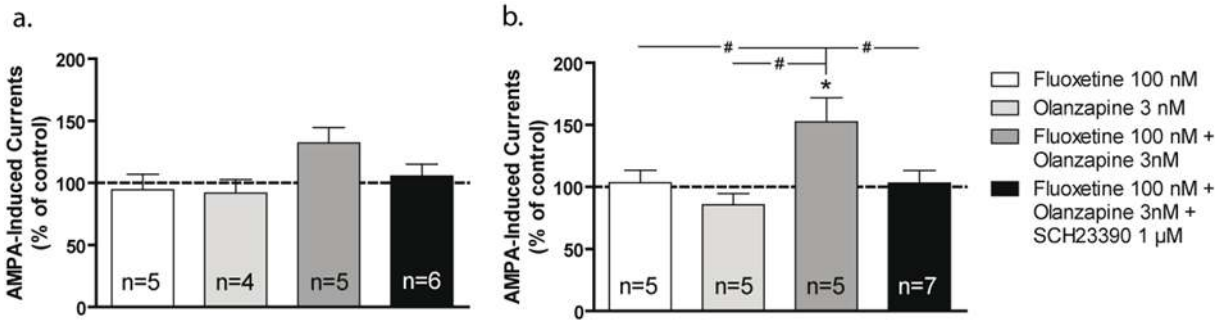

Figure 25. *A combination of olanzapine and fluoxetine facilitates AMPA-induced currents in pyramidal cells of the rat mPFC, via activation of the dopamine D_1 receptor. Bar charts showing the effect on AMPA-induced currents at (a) 5 min and (b) 30 min of drug administration. Data are presented as mean ± SEM (%). *p< 0.05 compared to control response. $^{\#}p<0.05$ indicates a between groups effect, as indicated in the figure.*

There was a trend for ketamine pretreatment towards enhancing the NMDA-induced (5 µM) currents, although this effect failed to reach statistical significance (figure 26a; p=0.0576). However, ketamine pretreatment significantly facilitated AMPA-induced (5 µM) currents (figure 26b).

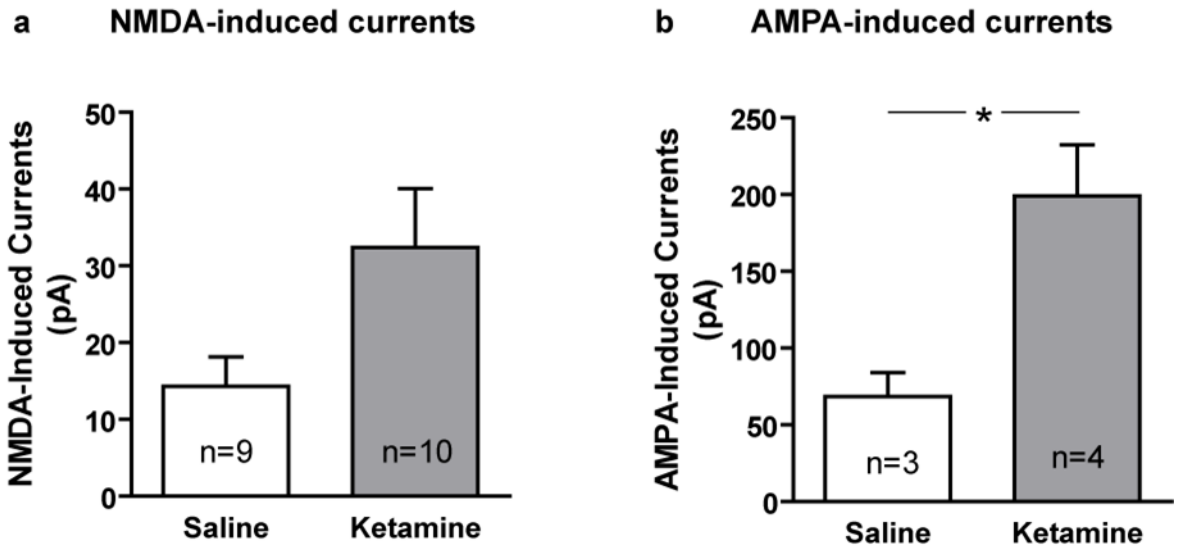

Figure 26. *Ketamine pretreatment enhanced AMPA-induced currents in pyramidal cells of the rat mPFC. (a) NMDA-induced currents in pyramidal cells from rats pretreated with ketamine (10 mg/kg) or saline (2 ml/kg). Bar chart shows mean± SEM. There was a trend for ketamine pretreatment to enhance the NMDA-induced currents but it failed to reach statistical significance (p=0.0576). (b) Ketamine pretreatment significantly enhanced AMPA-induced currents in pyramidal cells of the mPFC. *p<0.05 ketamine compared to saline. The number in each bar shows the groups size. The holding potential was -60 mV.*

4.3.3. Discussion: Effects of low doses of atypical antipsychotic drugs added to SSRIs on monoaminergic and glutamatergic neurotransmission in the mPFC.

Addition of low doses of asenapine to escitalopram enhanced the outflow of dopamine, noradrenaline and serotonin in the mPFC, indicating that asenapine may be effective as an adjunct in treatment-resistant depression, in similarity with e.g. olanzapine or quetiapine (c.f. 1.11.2). Asenapine is an antagonist at 5-HT_{2A}, α_2 and D_2 receptor and a partial agonist at the 5-HT_{1A} receptor, all of which may contribute to the increased monoamine release obtained when combined with the SSRI escitalopram. The increased monoamine outflow induced by this drug combination may stem from both systemic effects (Arborelius et al., 1993, Szabo and Blier, 2002, Ghanbari et al., 2009) as well as local mechanisms within the mPFC (Franberg et al., 2012). Clinical and preclinical studies suggest that α_2 and 5-HT_{2A} receptor antagonists can be used to potentiate the antidepressant or antidepressant-like effect of SSRIs (see e.g. Sanacora et al., 2004, Marek et al., 2005), further supporting the utility of asenapine as adjunct in MDD.

Using intracellular recordings in brain slices, we found that combinations of asenapine and escitalopram as well as of olanzapine and fluoxetine facilitated both NMDA and AMPA receptor-mediated transmission in the mPFC, via activation of the dopamine D_1 receptor. In similarity, injection of ketamine 24 hours prior to the electrophysiological experiment also produced a facilitation of AMPA receptor-mediated transmission, compared to saline treated rats. Moreover, ketamine pretreatment appeared to enhance also NMDA receptor-mediated transmission, although this effect did not reach statistical significance. Previous studies in rats have found ketamine pretreatment to enhance a number of synaptic proteins including the AMPA receptor subunit GluR1 and

to facilitate glutamatergic transmission in the mPFC and that the antidepressant-like effect of ketamine was abolished by a selective AMPA receptor antagonist (c.f. 1.11.1). Thus, the seemingly analogous results obtained with a combination of an atypical APD and an SSRI compared to the effect of ketamine in the present study, indicates that also the relatively rapid onset of the antidepressant effect obtained with a combination of atypical APDs and antidepressant drugs may be related to an enhancement of cortical AMPA receptor-mediated transmission. Further support for the notion that AMPA receptor-activation induces an antidepressant response is provided by studies showing that AMPA receptor allosteric modulators may exert a rapid antidepressant effect in animal models predictive of antidepressant effect (Li et al., 2001, Knapp et al., 2002).The mechanism by which the combinations of atypical APDs and SSRIs facilitate AMPA receptor-mediated transmission is not entirely clear. Although previous electrophysiological studies investigating the influence of D_1 activation on AMPA receptor-mediated transmission have generated conflicting results (see e.g. Tseng and O'Donnell, 2004, Smith et al., 2005), D_1 receptor activation has been found to increase and D_2 receptor activation to decrease the number of AMPA receptors on the cell surface of cortical pyramidal cells (Sun et al., 2005). Thus, enhanced dopamine outflow with concomitant blockade of the D_2 receptor, produced by the APD and SSRI, results in a preferential activation of D_1 receptors, which may result in an increase of the number of cell surface AMPA receptors. However, other mechanisms (e.g. serotonergic mechanisms) probably contributes to the facilitation of AMPA receptor-mediated transmission, since neither clozapine nor asenapine facilitates these currents when given alone, even though they facilitate NMDA receptor-mediated currents via the activation of the D_1 receptor at the same concentration (Arvanov et al., 1997, Jardemark et al., 2010). Tentatively, in the intact animal, ketamine may also, by augmenting the AMPA receptor-mediated transmission, secondarily enhance NMDA receptor-mediated transmission by reducing the voltage dependent Mg^{2+}-blockade.

Moreover, since the effect of ketamine seemed to be relatively more pronounced on AMPA receptor-mediated currents than on NMDA induced currents, our data are in principle consonant with previous findings and conclusions regarding the mechanism of action of ketamine (Maeng and Zarate, 2007).

Asenapine and escitalopram as well as a combination of olanzapine and fluoxetine facilitated NMDA receptor-mediated transmission via D_1 receptor activation. We also found that a combination of asenapine and escitalopram enhanced the area of the EPSPs and induced bursts of action potentials overriding the EPSPs in pyramidal cells of the rat mPFC, in similarity with results previously obtained with clozapine (Chen and Yang, 2002, Jardemark et al., 2005). This effect of clozapine was found to be dependent on NMDA and D_1 receptor activation. The action potentials induced by a combination of asenapine and escitalopram had varying onset latencies, indicating that they were elicited by recurrent activation of neighboring layer V pyramidal cells. This recurrent excitation of pyramidal cells in the PFC is thought to some extent explain the underlying physiological mechanism for working memory. Thus, low doses of

asenapine in combination with an SSRI may contribute to relieve cognitive deficits in e.g. depression. In addition to effects on memory, the activity of the NMDA receptor may also play a role in the antidepressant response *per se*, since drugs mediating their effect via the co-agonist site of the NMDA receptor (e.g. D-serine and a glycine reuptake inhibitors) have been found to generate an antidepressant effect in patients as well as in animal models predictive of antidepressant activity (Malkesman et al., 2012, Huang et al., 2013).

Ketamine, and other NMDA receptor-antagonists, has previously been found to increase dopamine release in the mPFC and D_1 receptor stimulation has been found to stimulate the mTOR pathway in the cortex (Schicknick et al., 2008). However, to which extent increased outflow in the mPFC induced by a combination of atypical APDs and an SSRI affect mTOR signaling, and to which extent dopamine-related effects of ketamine may contribute to its antidepressant effect remain to be determined.

In conclusion, we propose that the relatively rapid and enhanced antidepressant effect obtained when low to moderate doses of atypical APDs are added to SSRIs may result from a facilitation of monoamine outflow with ensuing facilitation of glutamatergic transmission in the PFC.

5. Summary and concluding remarks

Although considered as two separate diagnostical entities, increasing evidence points to a link between schizophrenia and depression. For example, depression is a common prodromal symptom of schizophrenia and it is estimated that the life-time prevalence of comorbid depression is 50% in schizophrenia and, *vice versa*, psychotic symptoms are more prevalent in patients diagnosed with depression as compared to the general public (Buckley et al., 2009). Depressive symptoms in schizophrenia are associated with poorer quality of life and worse long-term outcomes (Conley et al., 2007). The link between schizophrenia and depression is further supported by a recent study which showed that schizophrenia not only has a shared heritability with bipolar disorder (c.f. 1.9.1) but also with depression (Lee et al., 2013).

Moreover, the use of low doses of atypical APDs in non-psychotic depressed patients has been steadily increasing over the last decade. Results from a recent European study show that approximately 50% of the depressed in-patients in the study received an APD (Kasper, S., personal communication). Interestingly, the study also concluded that psychiatrists prescribed atypical APDs at moderate dosage as adjunct treatment to depressed patients to augment the antidepressant effect even long before regulatory authorities approved APDs for this indication.

In the present set of experimental studies we demonstrated that addition of the NET inhibitor reboxetine may further enhance the antipsychotic-like effect of a low but not a high dose of olanzapine, without increasing EPS liability. In parallel, adjunct reboxetine preferentially enhanced olanzapine-induced cortical dopamine output and facilitated NMDA receptor-mediated transmission in the mPFC. Similar results were obtained in a subsequent study when quetiapine was combined with the NET-inhibitor reboxetine. Moreover, we found that low doses of the novel atypical APD asenapine in combination with escitalopram enhanced monoamine output in the mPFC, and to some extent dopamine output in the NAc. Using electrophysiological intracellular recordings we found that a combination of low, clinically relevant concentrations of asenapine and escitalopram increased the area of electrically evoked EPSPs and facilitated the generation of action potentials in pyramidal cells of the rat mPFC.

Our data propose that concomitant NET-inhibition may allow for a lower D_2 receptor occupancy induced by the APD, yet with maintained antipsychotic effect. Concomitant NET inhibition may also ameliorate depressive and negative symptoms as well as cognitive impairments in schizophrenia by facilitating cortical dopaminergic transmission as well as NMDA receptor-mediated transmission. Moreover, our data propose that addition of reboxetine to olanzapine may allow for a dose reduction of olanzapine with maintained antipsychotic effect and an ensuing reduced risk of extrapyramidal side effects. Our data also suggest that NET inhibition, generated in patients by the active metabolite norquetiapine, may not only contribute to the antidepressant effect of quetiapine but, in addition, to the antipsychotic effect of quetiapine, that can be obtained in patients in spite of a low D_2 receptor occupancy.

We also showed that addition of asenapine to the SSRI escitalopram enhanced catecholamine output in the mPFC, in similarity with results obtained with olanzapine and fluoxetine (Zhang et al., 2000). In addition to the increased catecholamine output the combination of asenapine and escitalopram also facilitated serotonin output in the same brain region, in contrast to the effects obtained with olanzapine added to fluoxetine. This difference may be explained by the α_2-adrenoceptor antagonistic and 5-HT_{1A} partial agonistic properties of asenapine (Ghanbari et al., 2009, Franberg et al., 2012), receptors to which olanzapine have very low affinity (Schotte et al., 1996). This effect may be important, since an enhanced serotonin output may confer a therapeutic advantage in depression.

Asenapine and escitalopram as well as a combination of olanzapine and fluoxetine significantly potentiated NMDA receptor-mediated transmission via D_1 receptor activation in pyramidal cells *in vitro*, in similarity with the effects of clozapine. Given the importance of D_1 and NMDA receptor-mediated transmission in the mPFC for optimal cognitive function, this effect may contribute to ameliorate several aspects of cognitive dysfunctions in both schizophrenia and depression.

In subsequent electrophysiological experiments we showed that a combination of asenapine and escitalopram, as well as a combination of olanzapine and fluoxetine at low, clinically relevant concentrations, facilitates AMPA receptor-mediated transmission in pyramidal cells of the rat mPFC. Our data suggest that activation of the D_1 receptor may be necessary but probably not sufficient to facilitate AMPA receptor-mediated transmission. This effect was thus obtained by two different combinations of atypical APDs and SSRIs, proposing that facilitation of AMPA receptor-mediated transmission may represent a general effect of such drug combinations, in parallel with the enhanced antidepressant effect, which has been observed clinically with atypical APDs used as adjunct to SSRIs (Nelson and Papakostas, 2009). In support of this contention, our data demonstrate an enhanced AMPA receptor-mediated transmission in the mPFC following administration of a single dose of ketamine, 24 hours before (c.f. (Li et al., 2010), which has been found to generate a powerful antidepressant action with a fast onset of action.

In summary, our results propose that the rapidly augmented antidepressant effect obtained by adjunct treatment with low doses of atypical APDs in treatment-resistant depression maintained on conventional antidepressant drugs may be related to facilitation of monoamine outflow in the PFC with an associated facilitation of glutamatergic transmission.

Moreover, our results propose that asenapine may have potential clinical utility as adjunct treatment in treatment-resistant major depression, generating an enhanced antidepressant effect with a rapid onset. In fact, a clinical study investigating asenapine as adjunct to antidepressant drugs is currently ongoing (ClinicalTrials.gov Identifier: NCT01670019).

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