From the Department of Physiology and Pharmacology
Karolinska Institutet, Stockholm, Sweden

THE PATHOPHYSIOLOGICAL ROLES OF KYNURENIC ACID AND CYTOKINES IN PSYCHIATRIC ILLNESS

Sara Olsson

Stockholm 2011
Till mamma och pappa
ABSTRACT

Kynurenic acid is an astrocyte-derived tryptophan metabolite and a marker of neuroinflammation that antagonizes N-methyl-D-aspartate (NMDA) and α7 nicotinic acetylcholine receptors in the brain. Increased levels have been found in the cerebrospinal (CSF) and brains of patients with schizophrenia and experimental studies have shown that kynurenic acid bi-directionally influences dopaminergic neurotransmission. Hyperdopaminergia is suggested to underlie positive symptoms of schizophrenia and symptoms of mania/psychosis in bipolar disorder. Consistent with this notion, patients with schizophrenia show excessive amphetamine-induced dopamine release that correlates with the degree of positive symptoms. Further, patients with bipolar disorder show augmented amphetamine-induced behavioral response compared to controls. Here we examine the effects of elevated brain kynurenic acid on amphetamine-induced dopamine transmission, as well as the CSF content of kynurenic acid and cytokines in patients with bipolar disorder, in order to investigate their putative involvement in schizophrenia and bipolar disorder pathophysiology.

Accumbal dopamine release and ventral tegmental area (VTA) dopamine firing in response to amphetamine (2 mg/kg, i.p. or 0.2-25.6 mg/kg, i.v.) were measured by in vivo microdialysis with HPLC electrochemical detection and electrophysiology techniques. Sprague Dawley rats were treated with acute or subchronic L-kynurenine (5 mg/kg, s.c. or 90 mg/kg/day, s.c., for six days via osmotic minipumps) to elevate brain kynurenic acid levels. For locomotor activity experiments, C57BL/6 mice received acute or subchronic pretreatment of L-kynurenine (10 mg/kg, i.p. or 100 mg/kg, i.p., twice daily for six days). Spontaneous and amphetamine-induced (5 mg/kg, i.p.) locomotor activity was tested in a square open field arena.

The acute elevation of brain kynurenic acid resulted in increased anxiety-related behavior. Subchronic elevation of brain kynurenic acid produced an exaggerated amphetamine-induced accumbal dopamine release in the rat and increased locomotor activity in the mouse. These results might be related to the impaired amphetamine-induced feedback inhibition of VTA dopamine firing observed following subchronic elevation of brain kynurenic acid. Present results confirm that kynurenic acid modulates dopamine neurotransmission and behavior in rodents and that subchronic elevation of kynurenic acid is associated with dopaminergic changes that are consistent with findings in patients with schizophrenia or bipolar disorder.

Analysis of CSF showed increased levels of kynurenic acid and IL-1β, and decreased levels of IL-6, in patients with bipolar disorder. Positive correlations were found between the occurrence of recent symptoms of mania and the levels of IL-1β or kynurenic acid. Further, the lifetime occurrence of psychotic symptoms was associated with higher levels of kynurenic acid. Although causality needs to be determined, these results may suggest a pathophysiological role for IL-1β and kynurenic acid in psychiatric disorders involving symptoms of mania and psychosis. Given the present experimental results, prolonged elevation of brain kynurenic acid, possibly induced by increased levels of pro-inflammatory IL-1β, may cause a hyperdopaminergic state that drives symptoms of mania and/or psychosis. The positive correlation between CSF kynurenic acid and the dopamine metabolite homovanillic acid found in these patients further favor this idea.
LIST OF PUBLICATIONS


II. OLSSON SK, Larsson M and Erhardt S. Subchronic elevation of brain kynurenic acid augments amphetamine-induced locomotor response in mice. *Accepted for publication in Journal of Neural Transmission* DOI: 10.1007/s00702-011-0706-6


IV. OLSSON SK, Sellgren C, Engberg G, Landén M, Erhardt S. Cerebrospinal fluid kynurenic acid is associated with manic episodes and psychotic features in patients with bipolar disorder type I. *Manuscript*

# TABLE OF CONTENTS

1 Introduction ..................................................................................................................11
   1.1 Schizophrenia........................................................................................................11
      1.1.1 Prevalence and risk factors .................................................................11
      1.1.2 Symptoms.................................................................................................12
      1.1.3 Treatment .................................................................................................13
   1.2 Bipolar disorder.....................................................................................................13
      1.2.1 Prevalence and risk factors .................................................................14
      1.2.2 Symptoms -the bipolar spectrum .....................................................14
      1.2.3 Treatment .................................................................................................15
   1.3 Similarities and dissimilarities between schizophrenia and bipolar disorder ..16
   1.4 Hypotheses............................................................................................................18
      1.4.1 The dopamine hypothesis of schizophrenia ........................................18
      1.4.2 Dopamine in the pathophysiology of bipolar disorder ....................18
      1.4.3 The glutamate deficiency theory of schizophrenia .........................19
      1.4.4 Glutamate in bipolar disorder ............................................................20
      1.4.5 The kynurenic acid hypothesis of schizophrenia ............................21
      1.4.6 Theories of neuroinflammation in schizophrenia and bipolar disorder ..................................................................................................................23
   1.5 The dopamine system of the brain ......................................................................24
      1.5.1 Biosynthesis and elimination ................................................................25
      1.5.2 Receptors ..................................................................................................25
      1.5.3 Dopamine pathways ...............................................................................25
      1.5.4 Effects of d-amphetamine on dopamine neurons ............................26
   1.6 The glutamate system of the brain ......................................................................27
      1.6.1 NMDA receptors ....................................................................................27
   1.7 Kynurenic acid .....................................................................................................28
      1.7.1 Synthesis and regulation .......................................................................28
      1.7.2 Neurochemical properties ....................................................................31
   1.8 Immunological aspects .........................................................................................31
      1.8.1 Brain immune responses .......................................................................31
      1.8.2 Cytokines and behavior ........................................................................32
      1.8.3 Neuron-glia communication ....................................................................34
      1.8.4 Cytokines as neuromodulators .............................................................35

2 Specific aims of the study .........................................................................................36

3 Materials and Methods .............................................................................................37
   3.1 Animals and ethical aspects ...............................................................................37
   3.2 Drugs and chemicals ..........................................................................................37
   3.3 Elevation of endogenous kynurenic acid ..........................................................37
   3.4 Anesthesia and surgery for subchronic elevation of endogenous kynurenic acid (paper I and PET study) ..................................................................................38
   3.5 In vivo electrophysiology ...................................................................................38
      3.5.1 Anesthesia and surgery ..........................................................................38
      3.5.2 Recording electrode ................................................................................38
      3.5.3 Extracellular single cell recording .......................................................39
      3.5.4 Electrophysiological characteristics of dopaminergic neurons ..........39
4 Results and Discussion ................................................................. 50

4.1 The amphetamine-induced effects on dopamine transmission and behavior after acute and subchronic elevation of brain kynurenic acid in rodents ....... 50
  4.1.1 Effects on amphetamine-induced dopamine release (paper I) ...... 51
  4.1.2 Investigation of amphetamine-induced dopamine release in rat using small animal PET. ................................................. 52
  4.1.3 Effects on dopaminergic firing activity (paper I) .................... 52
  4.1.4 Effects on locomotor activity (paper II) .............................. 54
4.2 Cerebrospinal fluid kynurenic acid in patients with bipolar disorder .......... 56
  4.2.1 Kynurenic acid in patients and healthy volunteers (paper III) ...... 56
  4.2.2 Associations between kynurenic acid and symptoms of bipolar disorder (paper IV) ........................................... 58
  4.2.3 Additional results .................................................................. 59
4.3 Cerebrospinal fluid cytokines in patients with bipolar disorder ............... 60
  4.3.1 Cytokine profile and correlation with previous mood episodes (paper V) ............................................ 60
5 General Discussion ........................................................................ 62
6 Acknowledgements ........................................................................ 71
7 References ................................................................................. 74
## LIST OF ABBREVIATIONS

<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Full Form</th>
</tr>
</thead>
<tbody>
<tr>
<td>[¹¹C]RAC</td>
<td>[¹¹C]Raclopride</td>
</tr>
<tr>
<td>3-HK</td>
<td>3-hydroxykynurenine</td>
</tr>
<tr>
<td>α7nACH</td>
<td>α7 nicotinic acetylcholine</td>
</tr>
<tr>
<td>AMPA</td>
<td>α-amino-3-hydroxy-5-methyl-isoxazole-4-propionic acid</td>
</tr>
<tr>
<td>ANOVA</td>
<td>analysis of variance</td>
</tr>
<tr>
<td>AUC</td>
<td>area under the curve</td>
</tr>
<tr>
<td>cAMP</td>
<td>cyclic adenosine monophosphate</td>
</tr>
<tr>
<td>cf.</td>
<td>compare (	extit{confer} lat.)</td>
</tr>
<tr>
<td>CNS</td>
<td>central nervous system</td>
</tr>
<tr>
<td>COMT</td>
<td>catechol-\textit{O}-methyltransferase</td>
</tr>
<tr>
<td>COX</td>
<td>cyclooxygenase</td>
</tr>
<tr>
<td>CRP</td>
<td>C-reactive protein</td>
</tr>
<tr>
<td>CSF</td>
<td>cerebrospinal fluid</td>
</tr>
<tr>
<td>DAT</td>
<td>dopamine transporter</td>
</tr>
<tr>
<td>DOPAC</td>
<td>3,4-dihydroxyphenylacetic acid</td>
</tr>
<tr>
<td>e.g.</td>
<td>for example (\textit{exempli gratia} lat.)</td>
</tr>
<tr>
<td>EPS</td>
<td>extrapyramidal symptoms</td>
</tr>
<tr>
<td>GABA</td>
<td>γ-aminobutyric acid</td>
</tr>
<tr>
<td>GPR35</td>
<td>G protein-coupled receptor 35</td>
</tr>
<tr>
<td>GSK-3β</td>
<td>glycogen synthase kinase 3β</td>
</tr>
<tr>
<td>HAAO</td>
<td>3-hydroxyanthranilate 3,4-dioxygenase</td>
</tr>
<tr>
<td>HPLC</td>
<td>high-performance liquid chromatography</td>
</tr>
<tr>
<td>HVA</td>
<td>homovanillic acid</td>
</tr>
<tr>
<td>IDO</td>
<td>indoleamine 2,3-dioxygenase</td>
</tr>
<tr>
<td>i.c.v.</td>
<td>intracerebroventricular</td>
</tr>
<tr>
<td>i.e.</td>
<td>that is (\textit{id est} lat.)</td>
</tr>
<tr>
<td>IFN</td>
<td>interferon</td>
</tr>
<tr>
<td>IL</td>
<td>interleukin</td>
</tr>
<tr>
<td>IL-1R</td>
<td>interleukin-1 receptor</td>
</tr>
<tr>
<td>IL-1ra</td>
<td>interleukin-1 receptor antagonist</td>
</tr>
<tr>
<td>i.p.</td>
<td>intraperitoneal</td>
</tr>
</tbody>
</table>
i.v.  intravenous
ISI  interspike interval
IQ  intelligence quotient
KAT  kynurenine aminotransferase
Km  Michaelis-Menten constant
KMO  kynurenine 3-monooxygenase
KYNU  kynureninase
MAO  monoamine oxidase
MHC  major histocompatibility complex
NAD+  nicotinamide adenine dinucleotide
NF  nuclear factor
NMDA  N-methyl-D-aspartate
L-DOPA  L-3,4-dihydroxyphenylalanine
LTP  long-term potentiation
PAMP  pathogen-associated molecular patterns
PCP  phencyclidine
PFC  prefrontal cortex
PGE2  prostaglandin E2
PKA  protein kinase A
PKC  protein kinase C
PPI  prepulse inhibition
SARS  severe acute respiratory syndrome
s.c.  subcutaneous
SD  standard deviation
SEM  standard error of the mean
sIL-2R  soluble interleukin-2 receptor
SOC  suppressor of cytokine signaling
TDO  tryptophan 2,3-dioxygenase
TLR  Toll-like receptor
TNF  tumor necrosis factor
VTA  ventral tegmental area
1 INTRODUCTION

1.1 SCHIZOPHRENIA

Schizophrenia is a severe and disabling psychiatric disorder that profoundly affects the life of afflicted individuals and their relatives. The disorder typically strikes during late adolescence or early adulthood and is characterized by disturbances in cognition, emotional and social functioning, perception of reality and thought processing, which together with the poor prognosis of the disease have made it one of the world’s most important mental health issues. In addition, individuals with schizophrenia have high mortality rates (two to three times higher than in the general population) and their average life expectancy is reduced by about 20% (Auquier et al., 2007; Newman and Bland, 1991). Suicide is common among patients with schizophrenia, about ten times more common than in the general population, and is the single leading cause of excess deaths among these patients (Brown, 1997).

1.1.1 Prevalence and risk factors

The annual incidence (new cases) of schizophrenia has been quite stable over the past decades with an average of 15 per population of 100'000, and appears similar across continents and countries regardless of their economic status (McGrath et al., 2004; Saha et al., 2006). The point prevalence (existing cases at a given time point) is on average 4.5 per 1000 and the lifetime risk is about 0.7% (McGrath et al., 2008; Tandon et al., 2008). Contrary to previous beliefs, meta-analyses have revealed that factors such as urban living, a history of migration and male gender is associated with a higher risk for developing schizophrenia (Aleman et al., 2003; Cantor-Graae and Selten, 2005; McGrath et al., 2004; Pedersen and Mortensen, 2001). Despite decades of extensive research, the etiology and pathophysiology of this enigmatic disorder remains largely unknown. It is clear however, that both genetic and environmental factors are of importance for developing schizophrenia, and it has been estimated that genetic factors account for as much as 80% of the risk to develop the disease (Cannon et al., 1998; Cardno et al., 1999; Sullivan et al., 2003). Twin concordance rates are 0-28% and 40-50% for dizygotic and monozygotic twins, respectively (Cardno and Gottesman, 2000), and genetic studies have suggested a range of susceptibility genes, such as neuregulin, dysbindin, AKT1, DTNBP1, CHRNA7, RGS4, COMT and G72 (Law et al., 2006; Harrison and Weinberger, 2005). These genes are involved in neurotransmission, plasticity and synaptogenesis and might all contribute to the development of the disease. However, large variations and inconsistency of these findings limit our current understanding of this heterogenic genetic contribution. Epidemiological studies have highlighted several environmental risk factors for schizophrenia. For instance, malnutrition or maternal infection during the first half of pregnancy increases the risk, as does winter birth, older paternal age at conception, infection or trauma during childhood, cannabis abuse during adolescence and social stress (Allardyce and Boydell, 2006; Dalman et al., 2008; Davies et al., 2003; Harrison, 2004; Meyer et al., 2007;
Introduction

Morgan and Fisher, 2007; Penner and Brown, 2007; Read et al., 2005; Semple et al., 2005; Wohl and Gorwood, 2007).

1.1.2 Symptoms

Schizophrenia is a heterogeneous disease with large variations in symptomatology and treatment response among patients. The course is usually of chronic and relapsing nature with incomplete remissions. The symptoms are categorized in three broad clusters: positive symptoms, negative symptoms and cognitive deficits (see Andreasen, 1995). The positive (psychotic) symptoms are features added to normal behavior, such as hallucinations (mainly auditory), delusions and disorganized thought processes. The onset of positive symptoms typically occurs during late adolescence or early adulthood, between the age of 18 and 30, with about 5 years earlier onset in males (Angermeyer and Kuhn, 1988; Hafner et al., 1998). There is also a second peak of onset later in life among women. This first psychotic episode marks the formal onset of the disease and usually leads to the first contact with psychiatric care. The negative symptoms are characterized by the blunting or loss of normal functions, and include social withdrawal, anhedonia (inability to experience pleasure), alogia (poverty of speech), abulia (loss of motivation), apathy and lack of emotional responses. The negative symptoms are sometimes present several years prior to the first psychotic episode, during the prodromal phase, and usually become more prominent over the long-term course of the disease (Hafner and an der Heiden, 1999; Yung and McGorry, 1996). However, in contrast to the characteristic flattened emotional expression, patients often show increased emotional expression together with the positive symptoms, such as during emotional threatening hallucinations or persecutory delusions, a phenomenon known as the “emotional paradox of schizophrenia” (Aleman and Kahn, 2005). Cognitive deficits are present in almost all patients with schizophrenia and are usually moderately severe to severe compared to healthy individuals. Specifically, they include both impairments in discrete cognitive domains like verbal learning and memory, working memory, processing speed, executive function and sustained attention, and general impairment in global cognition and intelligence quotient (IQ; Irani et al., 2010; Kalkstein et al., 2010; Leeson et al., 2009b; Nuechterlein et al., 2004). Notably, cognitive deficits are also present in children who later develop schizophrenia and include impairments in intellectual, language, psychomotor and social functioning (Jones et al., 1994; Reichenberg et al., 2002; Woodberry et al., 2008). The cognitive and negative symptoms appear to be persistent over time, although a general cognitive decline is associated with the onset of illness (Mesholam-Gately et al., 2009; Sponheim et al., 2010). Positive symptoms are episodic and vary over the course of the disease. Predictors of poor outcome are male gender, early age of onset, prolonged period of untreated illness and severity of negative symptoms and cognitive deficits (Fenton and McGlashan, 1991; Leeson et al., 2009a; Perkins et al., 2005; Riecher-Rossler and Rossler, 1998).
1.1.3 Treatment

Since the discovery of the antipsychotic properties of chlorpromazine more than half a century ago (Delay et al., 1952), antipsychotic medication has constituted the primary pharmacological treatment for schizophrenia. The receptor binding profile varies among different antipsychotic compounds, but they all have one feature in common, the ability to block dopamine D$_2$ receptors in the brain. The first generation of drugs developed (e.g., chlorpromazine, haloperidol and fluphenazine) is usually referred to as typical antipsychotic drugs. While these compounds are effective in alleviating positive symptoms, which are believed to be mediated by dopaminergic hyperactivity in limbic areas, they have little or no effect on negative symptoms and cognitive deficits. Treatment with typical antipsychotics, drugs with high dopamine D$_2$ receptor occupancy in the brain, is unfortunately also associated with a number of extrapyramidal symptoms (EPS) such as motor control disturbances, like akathisia (inability to remain motionless), acute dystonia (involuntary sustained muscle contractions), parkinsonism (tremor, hypokinesia and rigidity) and the often irreversible tardive dyskinesia (involuntary stereotypical movements of the mouth, face and tongue; Casey, 1991). The severe side effects and lack of efficacy in treating negative symptoms and cognitive deficits prompted the development of a second generation of antipsychotic drugs known as atypical antipsychotics. The atypical antipsychotics (e.g., clozapine, olanzapine, risperidone and quetiapine), usually have lower dopamine D$_2$ receptor occupancy than typical antipsychotics and a broader receptor binding profile with high affinity also for serotonin receptors. Although atypical antipsychotics are considered less likely to cause EPS, they have other side effects such as weight gain, insulin resistance, increased risk of cardiovascular disease, sleep disturbance, extreme tiredness, hypersalivation, reduced sexual interest (Haddad and Sharma, 2007). As a consequence of these side effects, one major problem in the treatment of schizophrenia is medication nonadherence. Clozapine, the most efficient antipsychotic in treatment resistant schizophrenia is also associated with the severe albeit uncommon side effect agranulocytosis (Kane et al., 1988). Second generation antipsychotics are often marketed as having higher efficacy on the negative symptom spectra than first generation antipsychotic drugs, however, the evidence of an effect on negative symptoms independent from improvement on positive symptoms and EPS is poor. Due to the limited effectiveness of antipsychotic treatment on the negative symptoms and cognitive deficits of schizophrenia, other psychotherapeutic medications are readily used as adjunctive treatment. For instance, the anticonvulsant medications lamotrigine, valproic acid and carbamazepine are used to reduce aggressive or impulsive behavior. Antidepressants are used to reduce depressive and anxiety symptoms, benzodiazepines are used to alleviate symptoms of insomnia, agitation and anxiety and lithium to reduce affective symptoms.

1.2 BIPOLAR DISORDER

Bipolar disorder, also termed manic-depressive disorder, is a severely disabling and often life-threatening mental illness characterized by recurrent episodes of mania or hypomania, usually combined with episodes of depression. The various affective
episodes are separated by states of euthymia, when the mood is neutral and symptoms of mania or depression are absent. Onset usually occurs in late adolescence or early adulthood and the disease is almost always chronic. More than 70% of the patients experience recurrent episodes over a period of five years despite pharmacotherapy (Gitlin et al., 1995). Approximately 20% of patients experience four or more episodes within a year, a phenomenon termed rapid cycling (Calabrese et al., 1996; Schneck et al., 2004). Individuals with bipolar disorder have mortality rates about 2.5 times higher than the general population, and the rates for completed suicide are increased 15 and 22 times for males and females, respectively (Osby et al., 2001). With excess mortality, psychosocial impairment and occupational dysfunction the costs of bipolar disorder are high, both in terms of personal suffering and expense to society.

1.2.1 Prevalence and risk factors

The lifetime prevalence for bipolar disorder has been estimated as 0.6% for bipolar disorder I and 0.4% for bipolar disorder II (Merikangas et al., 2011). There is no major gender, race or ethnicity effect on the prevalence rate, although women are more likely to experience mixed states, depressive episodes and rapid cycling (Ketter, 2010). Bipolar disorder is highly heritable, with concordance rates of 57% for monozygotic twins and 14% for dizygotic twins (Alda, 1997), and the risk of developing a bipolar disorder is 13-fold increased in people with a first degree relative with the disease (Mortensen et al., 2003). Several susceptibility genes have been associated with bipolar disorder and, interestingly, a register-based epidemiological study suggests an overlap in genetic vulnerability with schizophrenia (Berrettini, 2003; Lichtenstein et al., 2009; see section 1.3). Moreover, psychosocial risk factors such as stressful life events have been demonstrated to increase relapse in bipolar disorder patients (Ellicott et al., 1990), and low social support is a predictor of bipolar disorder depression (Johnson et al., 1999). In addition, there is evidence to suggest that early parental loss, in particular maternal, is associated with an increased risk of developing bipolar affective disorder (Mortensen et al., 2003).

1.2.2 Symptoms -the bipolar spectrum

The bipolar spectrum of symptoms ranges from mild depression and hypomania to severe forms of depression and mania including psychosis. Due to this heterogeneity, bipolar disorder is divided into several subtypes. The current classification system acknowledges bipolar disorder type I, bipolar disorder type II, cyclothymia and bipolar disorder not otherwise specified. Bipolar disorder I is characterized by having at least one manic or mixed episode. The episode must last more than one week or require hospitalization. Elevated mood, euphoria, grandiose ideas, impulsivity, reduced need for sleep, increased talkativeness, racing thoughts, irritability and distractibility are characteristic manic symptoms. Manic episodes also commonly include psychotic symptoms like delusions or hallucinations (Dunayevich and Keck, 2000). The majority of individuals with bipolar disorder type I also experience a varying number of depressive episodes during their lifetime. Episodes of depression include a number of symptoms such as feelings of intense sadness, anxiety, guilt, loss of interest, fatigue,
feelings of worthlessness, insomnia, apathy and suicidal ideation or attempts that last for a period of at least two weeks. During a mixed episode, the afflicted individual experiences simultaneous symptoms of mania and depression. Hypomania is a state similar to mania but is less severe; the episode lasts for a shorter period and does not cause functional impairment (i.e., does not affect the social life or occupational situation of afflicted individuals). On the contrary, patients are often exceptionally creative, outgoing and productive during a hypomanic episode. Bipolar disorder II is defined by a history of at least one episode of hypomania in conjunction with at least one episode of depression, the more typical pattern being frequent depressions with one or more episodes of hypomania. Cyclothymia is defined by recurrent symptoms of hypomania and dysthymia that do not meet the criteria for a depressive or hypomanic episode. During a two-year period any symptom-free intervals last no longer than 2 months. Affected individuals are typically not severely functionally impaired. Instead, they are sometimes even over-productive. Bipolar disorder not otherwise specified, also known as sub-threshold bipolar disorder, describes bipolar disorder symptoms that do not meet the criteria for any of the other three diagnostic subtypes. In contrast with the disabling bipolar disorder I, the subdiagnoses bipolar disorder II and cyclothymia are often underdiagnosed. They are also commonly mistaken for unipolar depression since it is the depressive episodes that usually lead to contact with psychiatry.

In addition to affective symptoms, and as seen in patients with schizophrenia, patients with bipolar disorder also suffer from cognitive deficits, including impairments in verbal learning and memory, attention and executive function. Cognitive deficits are present from the onset of illness and in all states of the disease, including euthymia, although exacerbation generally occurs during acute episodes (Kurtz and Gerraty, 2009; Lewandowski et al., 2011; Martinez-Aran et al., 2004; Rubinsztein et al., 2000). Further, cognitive decline has been associated with the length of illness, number and length of episodes, in particular manic, and hospitalization (Cavanagh et al., 2002; Clark et al., 2002; van Gorp et al., 1998, for review see Robinson and Ferrier, 2006).

1.2.3 Treatment

The pharmacotherapy in bipolar disorder has two main objectives: 1) to treat an ongoing affective episode, termed acute treatment, and 2) to minimize the risk of recurrence and severity of future episodes and reduce symptoms between episodes, termed prophylactic treatment. Mood stabilizers are the main pharmacological treatment in bipolar disorder, and they are used both for acute treatment and to prevent future mood swings (manic, mixed or depressive episodes) without triggering episodes of the opposite polarity.

Commonly used mood stabilizers include lithium, anticonvulsants such as valproic acid, lamotrigine and carbamazepine, and atypical antipsychotics such as olanzapine, quetiapine and risperidone. Lithium is the most effective prophylactic treatment in bipolar disorder and prevents the recurrence of episodes to a high degree (for review see Grandjean and Aubry, 2009). Importantly, lithium also has an antisuicidal effect when used as prophylactic treatment (Schou, 2000; Tondo et al., 1997). Acute episodes of mania are typically treated with antipsychotics, but lithium, carbamazepine and valproic acid may also be used. With the exception of lamotrigine, mood stabilizers are
Introduction

usually less effective against symptoms of acute depression than acute mania. Combinations of antidepressants together with mood stabilizers are therefore often used during acute depressive episodes.

The mechanisms by which these drugs elicit their mood stabilizing effects are essentially unknown. However, drugs affecting the dopamine and serotonin transmission (atypical antipsychotics) efficiently alleviate symptoms of mania and psychosis. Anticonvulsants such as carbamazepine and lamotrigine reduce glutamatergic transmission while valproic acid increase γ-aminobutyric acid (GABA) levels. These effects are suggested to affect the afferent regulation of the mesolimbic dopamine system and, subsequently, the mood. In addition, lithium inhibits inositol monophosphatase, G-proteins, cyclic adenosine monophosphate (cAMP) and protein kinase A (PKA). Both valproic acid and lithium inhibit glycogen synthase kinase 3β (GSK-3β) and protein kinase C (PKC; Manji et al., 2001, for review see Rao and Rapoport, 2009), and many speculate that the mood stabilizing effects of these medications may arise from interference with signal-transduction pathways in neurons or surrounding glial cells. It has also been suggested that lithium, anticonvulsants and antipsychotics have specific cyclooxygenase (COX)-2 inhibiting and pro-inflammatory cytokine reducing effects that might contribute to their mood stabilizing properties (for reviews see Goldstein et al., 2009; Rao and Rapoport, 2009).

1.3 SIMILARITIES AND DISSIMILARITIES BETWEEN SCHIZOPHRENIA AND BIPOLAR DISORDER

Schizophrenia and bipolar disorder have many characteristics in common. They both have a lifetime risk of about 1% with typical onset in late adolescence or early adulthood. The disorders are often lifelong with an episodic course including periods of partial or complete remission. Both schizophrenia and bipolar disorder type I are psychotic disorders and acute mania including psychotic features can be indistinguishable from acute psychosis in schizophrenia. Moreover, manic and psychotic symptoms are believed to stem from hyperactivity in limbic areas and both respond to dopamine blockade. After onset, specific cognitive deficits in verbal learning and memory, attention and executive function are further core features in both disorders. Depression is common among patients with schizophrenia, and furthermore many of the negative symptoms characteristic of schizophrenia share clinical features with depression, such as social withdrawal and anhedonia. Both schizophrenia and bipolar disorder are highly heritable and family and twin studies have revealed a partial overlap in genetic susceptibility in the two disorders. Thus, the monozygotic co-twin of a person with schizophrenia has an increased risk for developing mania (8.2%) as well as schizophrenia (40.8%), while the monozygotic co-twin of a manic individual has an increased risk for schizophrenia (13.6%) as well as mania (36.4%; Cardno et al., 2002). In addition, monozygotic co-twins of schizoaffective individuals have equivalent increased risks of developing schizophrenia and mania (26.1%). Several chromosomal regions have been linked to increased risk for bipolar disorder and schizophrenia, including 10p14, 18p11, 13q32, 8p22 and 22q11 (Berrettini, 2003; Bramon and Sham, 2001) as well as a number of genes, including G72, BDNF and COMT (Chumakov et
al., 2002; Hattori et al., 2003; Kirov et al., 1998; Kunugi et al., 1997; Neves-Pereira et al., 2002; Rosa et al., 2006; Schumacher et al., 2004; Shifman et al., 2002).

Despite the many similarities regarding onset and course, symptoms, treatment and genetic susceptibility, there are a number of dissimilarities between the disorders as well. With schizophrenia, there is robust evidence for structural brain abnormalities. For instance, reduced whole-brain volume, enlarged ventricles and diminished gray matter in the temporal lobe, hippocampus and amygdala have been consistently found among these patients when compared to controls (Keshavan et al., 2008; Wright et al., 2000). In contrast to neurodegenerative disorders, the reduced brain volume observed in individuals with schizophrenia is not related to neural loss. Instead, defects have been demonstrated in brain structures associated with connectivity (i.e., the number of synapses, dendrites and axons, and glial cells; see Bernstein et al., 2009). Regarding bipolar disorder, the reports on brain morphology have been more inconsistent. Reduced whole brain volume is not reported compared to controls (Konarski et al., 2008). However, ventricular enlargement (+17%) has been observed, although to lesser degree than in schizophrenia (Kempton et al., 2008). A recent meta-analysis concluded that there are robust but regionally nonspecific alterations associated with bipolar disorder, and that morphological changes are more pronounced in patients with schizophrenia who show increased ventricular volume, reduced volume of the amygdala, and possibly increased globus pallidus volume compared to patients with bipolar disorder (Kempton et al., 2008). The reason for these structural differences is not clear, however it has been suggested that schizophrenia is associated with genes causing widespread gray matter development, while bipolar disorder is not (McDonald et al., 2004). In line with this notion, patients with bipolar disorder show less severe cognitive deficits than patients with schizophrenia, with no impairments in psychomotor and intellectual functioning (Altshuler et al., 2004; Keefe and Fenton, 2007; McIntosh et al., 2005). However, perhaps the most notable difference between schizophrenia and bipolar disorder is the premorbid functioning. Premorbid developmental abnormalities including the age of attaining developmental markers, impairments in social functioning, cognition, neurological and motor development and lower IQ are common among children who later develop schizophrenia but not in children who later develop bipolar disorder, who might even perform above average on some of these parameters (MacCabe et al., 2010; Messias et al., 2007). There are also notable dissimilarities regarding environmental risk factors, as schizophrenia has been associated with urban upbringing, winter birth, paternal age and prenatal infections. A population based study of 2.1 million Danish individuals failed to show such associations in bipolar disorder (Mortensen et al., 2003).

Taken together, many similarities exist in clinical characteristics, treatment regimens and genetic predisposition, indicating a partially shared pathophysiology between bipolar disorder and schizophrenia. However, our current knowledge on premorbid, etiological, and brain structural differences between the two disorders have led researchers to suggest that schizophrenia involves neurodevelopmental defects, while bipolar disorder does not.
1.4 HYPOTHESES

1.4.1 The dopamine hypothesis of schizophrenia

The dopamine hypothesis of schizophrenia has been the prevailing theory in schizophrenia research for the past 50 years. It originates from the discovery of antipsychotic drugs and their ability to increase dopamine metabolism in animals (Carlsson and Lindqvist, 1963; Delay et al., 1952). In its early version, an overall hyperactivity of dopamine transmission was proposed to be part of the pathophysiology in schizophrenia. The hypothesis was based primarily on pharmacological evidence. Specifically, the clinical effectiveness of antipsychotic drugs is related to their affinity to dopamine D$_2$ receptors (Creese et al., 1976; Seeman and Lee, 1975; Seeman et al., 1976), repeated use of dopaminergic drugs such as amphetamine can induce psychosis in healthy volunteers (Angrist et al., 1974) and worsen psychotic symptoms in patients with schizophrenia (Snyder et al., 1974), and further, reserpine (which effectively treats psychosis) blocks monoamine reuptake in vesicles leading to their elimination (Carlsson et al., 1957). Although this model had strong evidence that hyperdopaminergia was linked to symptoms of psychosis, negative symptoms and cognitive deficits were nevertheless resistant to dopamine D$_2$ receptor antagonism (Breier, 1999; King, 1998).

The emergence of more advanced brain imaging techniques eventually allowed researchers to investigate the brain functions of patients in vivo. Reduced blood flow was found in the prefrontal cortex (PFC) of patients with schizophrenia (Knable and Weinberger, 1997) that correlated with low cerebrospinal fluid (CSF) dopamine metabolite levels. Preclinical studies further revealed that dopamine D$_1$ receptors, primarily situated in the neocortex, were critical for PFC performance (Goldman-Rakic et al., 2000), while dopamine D$_2$ receptors were more abundant in subcortical regions. This led to a reformulation of the dopamine hypothesis where regionally specific dysregulation of dopamine pathways involving hyperdopaminergia in subcortical regions and hypodopaminergia in cortical regions were suggested to explain the full spectra of symptoms in schizophrenia (Davis et al., 1991).

More recently, brain imaging studies have provided additional evidence of a striatal hyperdopaminergia in patients with schizophrenia. One of the most consistent findings is increased presynaptic levels of dopamine, indicating enhanced synthesis of dopamine in these patients (Hietala et al., 1995; 1999; Howes et al., 2009; Lindstrom et al., 1999; McGowan et al., 2004; Meyer-Lindenberg et al., 2002; Reith et al., 1994). Further, patients with schizophrenia have approximately double the size of striatal dopamine release as compared with healthy volunteers following amphetamine challenge (Abi-Dargham et al., 1998; Breier et al., 1997; Laruelle and Abi-Dargham, 1999; Laruelle et al., 1996).

1.4.2 Dopamine in the pathophysiology of bipolar disorder

Despite decades of research a sufficient model for the pathophysiological mechanisms in bipolar disorder is still lacking. The mesocorticolimbic dopamine system is known to regulate functions such as emotional state, cognition, attention and reward, and
dysregulation of this system has been suggested to underlie the symptoms seen in bipolar disorder. Specifically, symptoms of mania and psychosis are believed to involve dopaminergic hyperactivity in limbic areas, consistent with the dopamine hypothesis of schizophrenia. Evidence for this theory include that dopaminergic drugs such as amphetamine and cocaine induce symptoms of euphoria, alertness, reduced need for sleep and over-confidence in healthy individuals, all of which are typical features of mania (Jacobs and Silverstone, 1986), and in higher doses or after repeated use they can also trigger psychosis (Angrist et al., 1974). Further, patients with bipolar depression are readily switched into hypomania when given L-3,4-dihydroxyphenylalanine (L-DOPA), the precursor of dopamine (Murphy et al., 1971), or dopamine agonists like bromocriptine or pramipexole (Aiken, 2007; Silverstone, 1984). In addition, antipsychotic medication, blocking dopamine D2 receptors, is effective in treating acute mania. Few brain imaging studies have been carried out on patients with bipolar disorder, but one study show increased behavioral response to the effects of amphetamine compared to healthy controls without any increase in striatal dopamine release, indicating an increased postsynaptic sensitivity to dopamine in these individuals (Anand et al., 2000). Conversely, hypodopaminergia has been considered with regard to the depressive symptoms of the disease. For instance, low CSF levels of the dopamine metabolite homovanillic acid (HVA) has been reported to be a marker for past potential lethality of suicide acts in bipolar and unipolar depressive patients (Agren, 1983), and for future suicide lethality in patients with bipolar disorder (Sher et al., 2006). Moreover, dopamine receptor agonists like bromocriptine and pramipexole can be used as antidepressants in bipolar disorder (Goldberg et al., 2004; Silverstone, 1984; Zarate et al., 2004b). In addition, cognitive processes regarding working memory have been linked to optimal prefrontal dopamine transmission, and both hypo- and hyperdopaminergic states are known to cause impairments in working memory (Abi-Dargham and Moore, 2003).

1.4.3 The glutamate deficiency theory of schizophrenia

The glutamate deficiency theory of schizophrenia proposes hypofunction in glutamatergic transmission as an underlying cause for the symptoms of schizophrenia. The theory is primarily based on the observation that dissociative anesthetics like ketamine and phencyclidine (PCP), drugs non-competitively antagonizing the N-methyl-D-aspartate (NMDA) receptor, induce schizophrenia-like symptoms, including positive and negative symptoms as well as cognitive deficits, in healthy individuals (Adler et al., 1999; Allen and Young, 1978; Krystal et al., 1994), and exacerbate symptoms in patients with schizophrenia (Lahti et al., 2001; Malhotra et al., 1997). Moreover, competitive NMDA receptor antagonists and drugs inhibiting the glycine binding site of the NMDA receptor have also been reported to produce psychotomimetic symptoms in humans (Albers et al., 1999; Grotta et al., 1995; Kristensen et al., 1992; Yenari et al., 1998). Inhibition of NMDA receptor function, by antagonizing the glycine, glutamate or non-competitive sites, induces increased midbrain dopamine activity in rodents (French, 1994; French et al., 1993; Linderholm et al., 2007). In healthy volunteers, ketamine causes increased levels of striatal dopamine (Breier et al., 1998; Vollenweider et al., 2000) and enhances the amphetamine-induced dopamine response (Kegeles et al., 2000).
Introduction

2000), thereby resembling the exaggerated amphetamine response observed in patients with schizophrenia (Abi-Dargham et al., 1998; Breier et al., 1997; Laruelle et al., 1996). Dysfunctional glutamate neurotransmission might thus underlie the dopaminergic aberrations observed in schizophrenia. There is also evidence that an impaired glutamate transmission could explain the hypothesized brain regional differences in dopamine transmission. While long-term NMDA receptor antagonism is associated with an increase in evoked striatal dopamine output in rodents (Balla et al., 2001; Jentsch et al., 1998b), it also causes reduced dopamine levels in PFC and cognitive impairments in both rats (Jentsch et al., 1997b; 1998a) and monkeys (Jentsch et al., 1997a; Tsukada et al., 2005). The exact mechanisms by which NMDA receptor antagonism alters dopamine transmission are not known, but an increased dopaminergic activity in limbic areas might be due to disinhibition of dopamine neurons, caused by a reduced afferent GABAergic influence on dopaminergic cell bodies in the ventral tegmental area (VTA; Zhang et al., 1993). Indeed, GABA transmission seems particularly vulnerable to impaired NMDA receptor signaling, as GABAergic interneurons are about 10-fold more sensitive to NMDA receptor inhibitors than glutamatergic neurons in the hippocampus (Grunze et al., 1996). Further support for the glutamate deficiency theory consist of genetic studies showing that polymorphisms of the NMDA subunit genes increase disease susceptibility (Itokawa et al., 2003; Ohtsuki et al., 2001; Rice et al., 2001), and postmortem findings showing abnormal NMDA receptor expression (Akbarian et al., 1996; Dracheva et al., 2001) and phosphorylation (Emamian et al., 2004) in patients with schizophrenia.

1.4.4 Glutamate in bipolar disorder

The glutamate system has been less extensively researched than the monoamine systems in bipolar disorder. However, mounting evidence suggest that glutamate signaling abnormalities are present in individuals with affective disorders and the recent discovery that ketamine has rapid (within hours) and potent anti-depressive effects has sparked an interest in the putative pathophysiological role of the glutamate system in various mood disorders.

There are a number of signs of altered glutamate transmission in bipolar disorder individuals. For instance, magnetic resonance spectroscopy studies have revealed increased cortical levels of glutamate and glutamine in all states of bipolar disorder (for review see Yuksel and Ongur, 2010). Further, elevated levels of glutamate have been found in the PFC or hippocampus of depressed (Frye et al., 2007b) and euthymic (Colla et al., 2009) individuals, as well as in postmortem brain of patients with bipolar disorder (Lan et al., 2009). However, conflicting data exists and decreased CSF levels of glutamate have, for instance, been reported in a cohort of mainly depressed unipolar and bipolar patients (Frye et al., 2007a). Alterations in glutamate receptor expression have also been observed. Thus, postmortem studies of patients with bipolar disorder have revealed reduced cortical expression of several NMDA receptor subunits including NR1, NR2B and NR3 (Beneyto et al., 2007; Beneyto and Meador-Woodruff, 2008; Rao et al., 2010), as well as reduced expression of cortical α-amino-3-hydroxy-5-methyl-isoxazole-4-propionic acid (AMPA) and kainate receptor subunits (Beneyto et al., 2007). However, the interpretation of some of the clinical
findings is complicated by medical influence, and the mood stabilizers lithium, valproic acid and carbamazepine for instance might all have downregulatory effects on the kainate receptor subunit GluK2 in mice astrocytes (Li et al., 2009).

A pathophysiological role for glutamate in bipolar disorder is also supported by pharmacological evidence. Several mood-stabilizing and anti-depressive drugs have shown the potential to affect glutamate signaling. The antiepileptic drugs carbamazepine, valproic acid and lamotrigine all affect glutamate transmission and are readily used as mood-stabilizers in the treatment of bipolar disorder (see section 1.2.3). Lithium was recently shown to postsynaptically attenuate both NMDA receptor and metabotropic glutamate receptor signaling in hippocampal neurons (Sourial-Bassillious et al., 2009), and chronic administration of several different classes of antidepressants is known to alter the subunit expression and function of NMDA receptors in rodents (Boyer et al., 1998; Nowak et al., 1996; 1998; Skolnick et al., 1996). Additionally, clinical trials have provided cogent evidence for a direct involvement of glutamate signaling in mood regulation. Thus, riluzole, a glutamate release inhibitor, has antidepressant effects both in patients with bipolar depression and major depression (Zarate et al., 2004a; 2005). Further, a single subanesthetic dose of ketamine has a very rapid and potent antidepressant effect in the treatment of resistant major depression (Berman et al., 2000; Zarate et al., 2006a), and in the treatment of resistant bipolar depression (Diazgranados et al., 2011). Interestingly, memantine, an NMDA receptor antagonist binding to the PCP-site with lower affinity than ketamine, has failed as a clinical antidepressant (Zarate et al., 2006b). Memantine has instead been shown to exert anti-manic effects in animal models (Gao et al., 2011), and is effective in treating psychotic symptoms in patients with Alzheimer’s disease (Clerici et al., 2011; Wilcock et al., 2008), and manic symptoms in patients with bipolar disorder (Keck et al., 2009; Koukopoulos et al., 2010). Although alterations in glutamate and NMDA receptor mediated neurotransmission seem to be involved in the pathophysiology of affective symptoms, the specific mechanisms involved still need further investigation.

1.4.5 The kynurenic acid hypothesis of schizophrenia

The kynurenic acid hypothesis of schizophrenia posits that elevation of the astrocyte-derived neuromodulator kynurenic acid causes alterations in glutamatergic, cholinergic and dopaminergic neurotransmission, ultimately leading to the symptoms of schizophrenia. The hypothesis originates from findings of elevated kynurenic acid in the CSF (Erhardt et al., 2001a) and in the postmortem cortex (Schwarcz et al., 2001) of patients with schizophrenia ten years ago. These initial reports were later confirmed by studies showing elevated kynurenic acid levels in the CSF of drug-naive and drug-treated patients (Nilsson et al., 2005), as well as increased levels of kynurenic acid and its precursor kynurenine in both CSF and cortical brain regions, as compared to healthy controls (Linderholm et al., 2010; Miller et al., 2006; Sathyasaikumar et al., 2010).

Kynurenic acid is mainly produced by astrocytes in the brain and has a unique receptor-binding profile with antagonistic properties both on the α7-nicotinic acetylcholine (α7nACh) receptor and the co-agonistic glycine site of the glutamatergic NMDA receptor (see section 1.7.2). The relevance of increased brain kynurenic acid in
schizophrenia pathophysiology is supported by several experimental findings. Hyperactivity in mesolimbic dopamine pathways might underlie the positive symptoms of schizophrenia and as with the exogenous NMDA receptor antagonists PCP and MK-801, elevation of brain kynurenic acid increases firing activity of midbrain dopamine neurons in rats (Erhardt and Engberg, 2002; Erhardt et al., 2001b; French, 1994; French et al., 1993; Linderholm et al., 2007; Nilsson et al., 2006). As kynurenic acid is an endogenous NMDA receptor antagonist that increases midbrain dopamine activity, the kynurenic acid hypothesis of schizophrenia is in agreement with the previously discussed dopamine and glutamate hypotheses. Notably, decreased levels of kynurenic acid leads to reduced firing of midbrain dopamine neurons (Schwieler et al., 2006; 2008), suggesting that dopamine activity is under the tonic control of brain kynurenic acid. Recent studies have further shown that kynurenic acid bi-directionally influences cortical and hippocampal glutamate release (Konradsson-Geukens et al., 2010; Pocivavsek et al., 2011), as well as modulates acetylcholine release (Zmarowski et al., 2009). Cognitive dysfunctions such as impaired working memory and verbal learning and memory are common in patients with schizophrenia and, in experimental studies, pharmacologically increased brain levels of kynurenic acid reduce cortical and hippocampal extracellular levels of glutamate and cause impairments in visuospatial working memory and contextual learning and memory in rodents (Chess and Bucci, 2006; Chess et al., 2007; 2009; Konradsson-Geukens et al., 2010; Pocivavsek et al., 2011). Further, prepulse inhibition (PPI), a behavioral test of sensorimotor gating, is impaired in both animals with elevated brain kynurenic acid (Erhardt et al., 2004), and in patients with schizophrenia (Baff et al., 2001). In contrast, lowering of endogenous brain kynurenic acid formation is associated with enhanced hippocampal plasticity and improved cognitive behavior (Potter et al., 2010).

Elevation of endogenous levels of brain kynurenic acid is likely related to increased formation of kynurenine, the immediate precursor of kynurenic acid. Increased availability of kynurenine could result either from inductions of tryptophan 2,3-dioxygenase (TDO) and/or indoleamine 2,3-dioxygenase (IDO), enzymes responsible for the rate-limiting step of the kynurenine pathway, or from reduced activity of kynurenine 3-monohydroxylase (KMO), an enzyme normally leading the precursor kynurenine down an alternate route of degradation (see section 1.7.1.1). Clinical data so far provide support for both of these events as increased levels of TDO (Miller et al., 2004; 2006) and decreased KMO activity (Sathyasaikumar et al., 2010) have been reported in patients with schizophrenia. The enzyme IDO can be induced by pro-inflammatory cytokines and increased levels of kynurenine and downstream metabolites of the kynurenine pathway are readily detected during infections in the central nervous system (CNS; see section 1.7.1.3). In addition, unpublished data from our laboratory reveal that TDO and IDO gene expression, along with increased kynurenic acid synthesis, can be induced by the pro-inflammatory cytokine interleukin (IL)-1β in cultured human astrocytes (manuscript to be submitted). Increased levels of CSF IL-1β have recently been reported in first episode patients with schizophrenia, thus supporting a possible cytokine-mediated increase in brain kynurenic acid in these patients (Soderlund et al., 2009). Interestingly, selective COX-2 inhibitors (eg., celecoxib and parecoxib) are known to decrease the formation of brain kynurenic acid in rats resulting in reduced dopaminergic activity (Schwieler et al., 2005; 2006; 2008), as well as to improve symptoms of schizophrenia, bipolar disorder and major depression when given as adjunctive treatment (Akhondzadeh et al., 2007; 2009; Muller et al., 2002; 2006; 2010; Nery et al., 2008). Conversely, selective inhibitors of
the constitutively expressed enzyme COX-1 give rise to both increased brain kynurenic acid and dopaminergic activity in rodents (Schwieler et al., 2005; 2006; 2008) and are associated with psychotic side-effects in humans (Clunie et al., 2003; Hoppmann et al., 1991; Tharumaratnam et al., 2000).

1.4.6 Theories of neuroinflammation in schizophrenia and bipolar disorder

There are two foundations for an “inflammatory theory” of schizophrenia. One, and perhaps the most frequently discussed, is the association between prenatal and early life infections and the increased risk of developing schizophrenia in adulthood. Various infections have been shown to be of interest in this regard. For instance, toxoplasmosis, rubella and influenza have been demonstrated to increase the lifetime risk of schizophrenia (Brown et al., 2001; 2005; Mednick et al., 1988). Since the infectious insult occurs during brain development and lacks pathogen specificity, it has been suggested that common immune response mediators, such as cytokines, might interfere with normal brain development. The second basis for an inflammatory etiopathophysiology was established based on early reports of schizophrenia-like behavior among influenza pandemics (Goodall, 1932; Menninger, 1928). Although it is now known that schizophrenia is not caused by acute encephalitis, Menninger’s observations, together with more recent clinical reports of psychiatric symptoms among patients with severe acute respiratory syndrome (SARS; Cheng et al., 2004; Sheng et al., 2005), have triggered speculations that neuroinflammation might precipitate symptoms of psychosis in genetically predisposed individuals. Indeed, corona-virus immunoreactivity has recently been associated with acute psychotic symptoms (Severance et al., 2011). Further, neuropsychiatric symptoms including cognitive deficits, mood symptoms and psychosis are common among patients with the autoimmune disease systemic lupus erythematosus, and the occurrence of psychosis in these patients has been linked to increased CSF levels of the pro-inflammatory cytokines interferon (IFN)-α and IL-6 (Hirohata et al., 2009; Hirohata and Miyamoto, 1990; Shiozawa et al., 1992). Numerous studies have shown peripheral cytokine changes in patients with schizophrenia. These include alterations in IL-1β, IL-1 receptor antagonist (IL-1ra), tumor necrosis factor (TNF), IL-6, IFN-γ, IL-2, and IL-10 and various cytokine receptors. However, there are conflicting data and the most consistent results show increased serum or plasma levels of IL-1ra, soluble IL-2 receptor (sIL-2R) and IL-6 (Potvin et al., 2008). Fewer studies have examined the cytokine profile in the brain or CSF of patients with schizophrenia. However, a recent study examining a broad range of cytokines in the CSF of first episode patients showed an isolated and drastic increase in the pro-inflammatory cytokine IL-1β compared to healthy volunteers (Söderlund et al., 2009). With regard to the previously discussed dopamine hypothesis of schizophrenia, it is of particular interest that elevation of brain IL-1β decreases PFC levels of dopamine, and causes impaired memory and anxiety-related behavior in rats (Song et al., 2006; 2008). In further support for a pro-inflammatory pathophysiology, antipsychotic drugs are known to exert immunosuppressant effects. Thus, chlorpromazine has been found to reduce the production of the pro-inflammatory cytokines TNF and IL-1β and increase anti-inflammatory IL-10 in mouse plasma/serum (MengoZZi et al., 1994; Netea et al., 1995), while haloperidol decreases TNF and IL-1β in human monocytes (Kowalski et al.,
In contrast to schizophrenia, prenatal or early life infections have not been associated with increased risk of developing bipolar disorder. However, evidence indicating ongoing neuroinflammatory processes have been reported in these patients. Alterations in the plasma levels of cytokines and acute phase proteins have been reported during all states of the disease (e.g., increased levels of C-reactive protein [CRP], TNF, IL-6, IL-8 and sIL-2R during both mania and depression, and increased sIL-2R and decreased IL-6 and IL-10 in euthymic patients; for review see Goldstein et al., 2009). Evidence of inflammatory processes within the brain has also recently been reported in a postmortem study showing increased protein and mRNA levels of IL-1β, the IL-1 receptor (IL-1R), the immune-regulating nuclear factor (NF)-κB and astroglial and microglial markers (Rao et al., 2010). Moreover, pro-inflammatory cytokines cause sickness-behavior, a motivational state similar to depression (see section 1.8.1). In line with this, immunotherapy with IFNs in cancers, viral infections or autoimmune disorders is associated with the induction of symptoms of depression, but also various symptoms like irritability, hypomania, mania, mixed states, psychosis, cognitive deficits, delirium and anxiety (Capuron et al., 2002; Capuron and Miller, 2004; Constant et al., 2005; Fragoso et al., 2010; Giunta et al., 2007; Raison et al., 2005). Investigations regarding the mechanism of action of mood stabilizers provides additional support for a pro-inflammatory pathophysiology in bipolar disorder as lithium, valproic acid and lamotrigine all mediate anti-inflammatory effects with reduced levels of COX-2 and prostaglandin E2 (PGE2) in the rat brain as a result of treatment (Bosetti et al., 2002; Lee et al., 2008; Rao et al., 2007, for review see Berk et al., 2011). Lithium is further shown to downregulate NF-κB in rat brain (Rao et al., 2007) and valproic acid to inhibit TNF and IL-6 production, and NF-κB activation in human monocytes (Ichiyama et al., 2000).

1.5 THE DOPAMINE SYSTEM OF THE BRAIN

Dopamine is a catecholamine and the precursor of the neurotransmitters norepinephrine and epinephrine. Carlsson and coworkers were first to recognize dopamine as a neurotransmitter in its own right in the late 1950s (Carlsson et al., 1957; 1958). Since then, immunohistochemistry and tract-tracing techniques have enabled researchers to map the dopamine system, making it one of the most well-characterized neurotransmitter systems in the brain. The physiological role of dopamine in the brain is diverse and involves behavior, mood, memory, attention, learning, motivation, reward, motor control and endocrine activity, and aberrations in the dopaminergic system have been implicated in a number of brain disorders (e.g., Parkinson’s disease, attention deficit disorder, psychosis, schizophrenia, mood disorders and drug dependence).
1.5.1 Biosynthesis and elimination

Dopamine is synthesized in dopaminergic neurons from the precursor amino acid L-tyrosine. The enzyme tyrosine hydroxylase, which is present in all catecholamine-containing cells, is the rate-limiting enzyme in dopamine synthesis and converts L-tyrosine to L-DOPA. L-DOPA is in turn decarboxylated into dopamine by the enzyme aromatic L-amino acid decarboxylase. The dopamine is packed and stored in cytosolic vesicles and released into the synaptic cleft by fusion of the vesicle with the cellular membrane. The vesicular release of dopamine is Ca\(^{2+}\)-dependent and triggered by action potential-induced membrane depolarization that opens voltage sensitive Ca\(^{2+}\) channels situated in the terminal region of the neuron. In the synaptic cleft dopamine is eliminated either by reuptake via the dopamine transporter (DAT) and further catabolized by intraneural monoamine oxidase (MAO) to dihydroxyphenylacetic acid (DOPAC), or via extracellular degradation by catechol-O-methyltransferase (COMT) to 3-methoxytyramine which in turn is converted to HVA by MAO.

1.5.2 Receptors

Dopamine receptors are G-protein coupled and mediate slow synaptic transmission through intracellular changes of second messengers. There are currently five identified dopamine receptors, which are divided into two subtypes: D_{1}-type and D_{2}-type receptors. The D_{1}-type receptors, D_{1} and D_{5}, are primarily coupled to Go_{S} and stimulate the production of cAMP. The D_{2}-type receptors (D_{2}, D_{3} and D_{4}) are Go_{i/o} coupled and inhibit the production of cAMP (Jaber et al., 1996). D1 receptors are the most widespread dopamine receptors of the brain and are known to be of major importance for cognitive functioning (Abi-Dargham and Moore, 2003; Goldman-Rakic et al., 2004). While both dopamine D_{1}- and D_{2}-type receptors can be found pre- and postsynaptically, the D_{2}-type receptors expressed on presynaptic and somatodendritic regions act as autoreceptors, and are important for negative feedback and control of dopaminergic activity (Cameron and Williams 1993; Pennartz et al., 1992; Stoof and Kebabian, 1984; White, 1996; White and Wang, 1984).

1.5.3 Dopamine pathways

There are four major dopaminergic pathways in the mammalian brain: the nigrostriatal, the mesolimbic, the mesocortical, and the tuberoinfundibular pathways. In addition, a fifth pathway called the “thalamic dopamine system”, which appears to be specific for humans and non-human primates, has been described (Sanchez-Gonzalez et al., 2005). The mesolimbic and mesocortical pathways are involved in brain functions regarding behavior, emotional control, memory, cognition, reward and motivation. In these pathways the dopaminergic cell bodies are situated in the VTA, located in the midbrain, and project either to limbic structures such as the ventral striatum, hippocampus and amygdala (mesolimbic pathway) or the prefrontal cortex (mesocortical pathway; Guillen et al., 2007; Koob and Swerdlow, 1988; Moore and Bloom, 1978; Ungerstedt, 1971). Specifically, the ventral striatum (including the nucleus accumbens) is involved in motivation, emotional control and behavior, which are functions of particular interest.
for bipolar disorder and schizophrenia, and induce locomotor behavior in rodents (Koob and Swerdlow, 1988). Increased activity in mesolimbic dopamine neurons, is believed to mediate symptoms of mania and psychosis, while decreased transmission in the mesocortical pathway is associated with problems in memory and cognition, and a dysregulation of the mesolimbic and mesocortical systems has been suggested in the pathophysiology of schizophrenia (see section 1.4.1). The nigrostriatal pathway originates from substantia nigra pars compacta and projects to the dorsal part of striatum via the medial forebrain and the internal capsule (Anden et al., 1964; Carlsson, 1959). This part of the dopamine system is of major importance for motor control and coordination, and degeneration of nigrostriatal dopamine neurons is associated with Parkinson’s disease (Hornykiewicz and Kish, 1987). In addition, EPS side effects caused by antipsychotic medication arise from inhibition of this system. The tuberoinfundibular pathway is involved in endocrine control. It originates in the arcuate nucleus of the hypothalamus and inhibits the secretion of prolactin from the anterior pituitary by the release of dopamine into the portal vessels. The thalamic dopamine system differs from other dopamine pathways as it involves dopamine cells located in multiple sites e.g., the periaqueductal gray matter, the ventral mesencephalon, the hypothalamic nuclei and lateral parabrachial nucleus, that all project to the thalamus. The physiological function of this pathway is unknown, but it has been suggested to be involved in gating information transfer between the thalamus, neocortex, striatum, and amygdala (Sanchez-Gonzalez et al., 2005).

### 1.5.4 Effects of d-amphetamine on dopamine neurons

Amphetamine is a psychostimulant drug that through the release of norepinephrine, dopamine and serotonin produces symptoms such as increased alertness and focus, euphoria, increased self-confidence, sociability, irritability, grandiosity, reduced need for sleep, decreased fatigue and decreased appetite. High doses or repeated use can also induce amphetamine psychosis (Angrist et al., 1974).

Upon systemic administration, the lipophilic amphetamine enters the brain where it increases extracellular dopamine by a number of mechanisms. Unlike cocaine, which prevents the cellular uptake of dopamine by inhibiting DAT, amphetamine competes with dopamine for uptake by DAT and interferes with the transporter causing a reversed DAT-mediated efflux of dopamine (Fischer and Cho, 1979; Fleckenstein et al., 2007; Kahlig and Galli, 2003). In addition, amphetamine can diffuse into the dopamine storing vesicles in the nerve terminal. Being a weak base with a pKa of 9.9, amphetamine is thought to be swiftly protonized and trapped in the acidic milieu of the vesicles leading to accumulation of amphetamine and eventually disruption of the membrane proton gradient. Vesicles with a disrupted proton gradient lose their ability to store dopamine leading to increased cytosolic concentration of dopamine, which then exits the cell by reverse transport via DAT (Fleckenstein et al., 2007; Sulzer et al., 1995). Further dopaminergic actions of amphetamine include inhibiton of MAO, leading to reduced dopamine breakdown in the cytosol, and stimulation of dopamine synthesis (Green and el Hait, 1978; Uretsky and Snodgrass, 1977). The large amount of extracellular dopamine released by amphetamine stimulates dopamine D1-type receptor activated long-loop regulatory feedback pathways (Bunney and Aghajanian 1978). Further, denritic dopamine release stimulates D2 autoreceptors located on somal and dendritic membranes, as well as D1 receptors on GABAergic afferents impinging on
dopamine cell bodies, leading to inhibition of dopamine cell firing (Shi et al., 2000; White 1996). Thus, dual effects on dopamine transmission accompany amphetamine administration with a simultaneous increase in dopamine release as well as a reduction of dopamine cell firing.

1.6 THE GLUTAMATE SYSTEM OF THE BRAIN

Glutamate is the most abundant excitatory neurotransmitter in the mammalian brain. It is involved in most brain functions and is crucial for synaptic plasticity, learning and memory (McEntee and Crook, 1993). In the brain, glutamate is synthesized from glutamine, which is transported into the brain across the blood-brain barrier. Glutamate is stored in synaptic vesicles and released via exocytosis triggered by action potential-mediated Ca\(^{2+}\) influx. Specific glutamate transporters situated on neurons and adjacent astrocytes rapidly clear released glutamate from the synaptic cleft via an energy-dependent transport process (Danbolt, 2001). Glutamate is subsequently converted to glutamine inside the astrocyte and released back to the glutamatergic neuron. Further, astrocytic end-feet are co-localized with pre- and postsynaptic glutamatergic elements and astrocytes are recognized as an integral part of glutamatergic synaptic transmission, a concept in synaptic physiology termed the tripartite synapse (Perea et al., 2009). In addition to regulating glutamate uptake and recycling, astrocytes monitor and modulate glutamate transmission by releasing a number of neurotransmitters and neuromodulators (see section 1.8.3).

Glutamate receptors belong to two functionally different families: ionotropic receptors, which are ion channel receptors called NMDA, AMPA and kainate receptors, and metabotropic receptors, which are G-protein coupled receptors that are generally divided into three types according to their intracellular activity. Glutamate is the endogenous ligand for all glutamate receptors, but also the amino acid aspartate can, to a lesser extent, function as a ligand for these receptors. Overstimulation of ionotropic glutamate receptors, particularly the NMDA receptor, can cause excitotoxicity by allowing large quantities of Ca\(^{2+}\) into the cell. The intracellular Ca\(^{2+}\) then activates enzymes leading to cell damage and even cell death (Meldrum and Garthwaite, 1990).

1.6.1 NMDA receptors

The NMDA receptor is a complex ion channel receptor that is nonselective for cations with high permeability for Ca\(^{2+}\). NMDA receptors are unique in that they are both voltage- and ligand-gated. Mg\(^{2+}\) binds to the inside of the ion channel and physically blocks cation influx (even in the presence of glutamate) until removed by a depolarization of the membrane potential. This depolarization and release of Mg\(^{2+}\) block is typically mediated by AMPA receptor activation (McBain and Mayer, 1994). Moreover, a co-agonistic allosteric D-serine/glycine-binding site regulates channel open time and desensitization rate, and several additional regulatory sites sensitive to Zn\(^{2+}\), protons and reduction/oxidation agents have been reported (Danysz and Parsons, 1998; McBain and Mayer, 1994). The complex voltage- and ligand-dependent regulation of NMDA receptors makes it dependent on the accumulated synaptic input.
This gives NMDA receptors a unique feature that is essential for its role in synaptic plasticity. Synaptic stimuli resulting in the activation of NMDA receptors leads to a long-lasting increase in the synaptic efficacy, a process called long-term potentiation (LTP), which is believed to be of major importance for learning and memory functions (Lynch, 2004).

The NMDA receptors form heteromeric complexes composed of the NR1 subunit together with NR2 (NR2A-D) or NR3 (NR3A-B) subunits. The NR1 subunit contains the glycine binding site while the NR2 subunit contains the glutamate binding site (Monaghan and Jane, 2009). The NR3 subunit can assemble with NR1 to form a glycine-activated channel complex unaffected by glutamate or NMDA (Chatterton et al., 2002). Interestingly, these glycine-activated receptors are inhibited by D-serine, while at conventional NDMA receptors both D-serine and glycine acts as co-agonists. Thus, the receptor composition and the availability of D-serine/glycine seem crucial for NMDA receptor function.

1.7 KYNURENIC ACID

Tryptophan is an essential amino acid in the human diet that is central for protein synthesis and acts as the precursor for serotonin and several neuroactive compounds. One of these compounds, kynurenic acid, was first discovered 1853 in canine urine (Liebig, 1853) and was later recognized as an end metabolite of tryptophan produced along the catabolic kynurenine pathway (Beadle et al., 1947; Heidelberger et al., 1949; see figure 1). In most mammalian tissues, including the brain, the kynurenine pathway is the major route of tryptophan degradation. Kynurenic acid is neuroactive and modulates glutamate, dopamine and acetylcholine neurotransmission. Although its physiological role is not fully understood it is known to be anticonvulsive and protect against neurotoxicity, as well as influence dopamine activity, cognition and sensorimotor gating functions in animals (for review see Erhardt et al., 2009).

1.7.1 Synthesis and regulation

1.7.1.1 The kynurenine pathway

The initiating step in the kynurenine pathway is the oxidation of tryptophan to N-formylkynurenine, catalyzed by the enzymes IDO or TDO. N-formylkynurenine is then rapidly hydrolyzed to kynurenine by kynurenine formamidase. Kynurenine is the central compound of the kynurenine pathway and can be readily transported across the blood-brain barrier by the large neutral amino acid carrier. There are three different routes by which kynurenine can be further catabolized: (i) by kynurenine aminotransferases (KAT I, KAT II, KAT III and KAT IV) to the end metabolite kynurenic acid, (ii) by KMO to 3-hydroxykynurenine (3-HK) or (iii) by kynureninase (KYNU) to anthranilic acid. 3-HK is a free radical generator (Stone, 1993) that together with anthranilic acid is further catabolized to 3-hydroxyanthranilic acid and subsequently 2-amino-3-carboxymuconic semialdehyde, which in turn is converted to quinolinic acid, an excitotoxic NMDA receptor agonist. Quinolinic acid is further catabolized via nicotinamide to nicotinamide adenine dinucleotide (NAD+). Although all of the kynurenine pathway enzymes are expressed in brain tissue, the route of
kynurenine degradation varies between different cell types. Astrocytes have been found to mainly express KAT enzymes while lacking KMO, thereby leading the kynurenine breakdown towards kynurenic acid production (Guidetti et al., 2007b; Guillemin et al., 2001; Kiss et al., 2003). In contrast, microglia have limited expression of KAT enzymes and are the main source of quinolinic acid production in the brain (Lehrmann et al., 2001). Availability of enzymes thus create a spatial separation between the two branches of the kynurenine pathway leading towards the neuroprotective kynurenic acid and the excitotoxic quinolinic acid synthesis. Moreover, recent evidence show that kynurenic acid also can be produced from D-tryptophan or D-kynurenine via the enzyme D-amino acid oxidase (DAAO) in the mammalian brain, and that this metabolic route can contribute to brain kynurenic acid production in vivo (Ishii et al., 2011; Ogaya et al., 2010).

**Figure 1.** The kynurenine pathway

### 1.7.1.2 Brain KAT enzymes

Because of its poor blood-brain crossing ability, kynurenic acid has to be produced inside the brain. The synthesis of kynurenic acid involves an irreversible transamination of kynurenine, and four enzymes capable of this conversion have been discovered in mouse, rat and human brains: KAT I, KAT II, KAT III and KAT IV (also known as mitochondrial aspartate aminotransferase; Han et al., 2010).
Introduction

KAT I and II were the first discovered and are expressed mainly by glial cells (Buchli et al., 1995; Okuno et al., 1990; 1991a; 1991b; Vezzani et al., 1990). Both enzymes are believed to contribute to kynurenic acid formation in the brain, however differences in pH optimum (pH 9.5 to 10 for KAT I and pH 7.4 for KAT II) and higher substrate specificity of the KAT II enzyme suggest that KAT II is the main catalyst under physiological conditions (Guidetti et al., 1997; Schmidt et al., 1993). It has been estimated that KAT II accounts for as much as 75% of the brain production of kynurenic acid in the rat (Guidetti et al., 1997). However, regional differences may occur and KAT I is about 55 times more abundant than KAT II in the rat cerebellum (Guidetti et al., 1997). Two additional enzymes, KAT III and KAT IV, are able to catalyze the transamination of kynurenine to kynurenic acid (Guidetti et al., 2007a; Yu et al., 2006), however, it remains to be investigated if these enzymes contribute to brain kynurenic acid formation under physiological conditions in vivo.

1.7.1.3 Regulation of kynurenic acid synthesis

KAT I and KAT II have Michaelis-Menten constants (Km) in the millimolar range indicating that kynurenine availability is the rate limiting factor in kynurenic acid synthesis (Han et al., 2008; Han and Li, 2004; Passera et al., 2011). Experimentally, increased levels of brain kynurenic acid can be achieved either by administering kynurenine (Jauch et al., 1993; Swartz et al., 1990; Wu et al., 1992) or by blocking KMO activity, the enzyme converting kynurenine to 3-HK, thus shunting the production towards kynurenic acid (Carpenedo et al., 1994; Speciale et al., 1996). Further, kynurenic acid is an end metabolite that is renally excreted after being eliminated from the brain via probenecid sensitive transporters (Moroni et al., 1988; Turski and Schwarcz, 1988). Elevation of endogenous brain kynurenic acid can therefore also be achieved by probenecid administration (Moroni et al., 1988).

The rate-limiting step in the production of kynurenic acid is the conversion of tryptophan to N-formylkynurenine by IDO or TDO. While IDO is widely distributed in various tissues such as lungs, intestines, placenta and brain, the vast majority of TDO is expressed in peripheral organs such as liver and kidney (Stone, 1993), and peripheral kynurenine is the main source (60-78%) of brain kynurenic acid (Gal and Sherman, 1978; Kita et al., 2002). Pro-inflammatory cytokines such as IFNs are known to induce IDO and additional cytokines (e.g., IL-1β, IL-4, IL-6, IL-10, PGE2, TGFβ, and suppressor of cytokine signaling [SOC] 3), have also been shown to modulate IDO expression (for reviews see Hayley, 2011; King and Thomas, 2007). KAT I and KAT II expression is also induced by IFN-γ (Guillemin et al., 2001) and preliminary data from our laboratory show that IDO and TDO expression can be induced by the pro-inflammatory cytokine IL-1β in cultured human astrocytes (unpublished data). Thus, the production of kynurenine and subsequently of kynurenic acid, is highly influenced by immunological factors and increased CSF levels of kynurenine and downstream catabolites have been observed in humans during various CNS infections (Atlas et al., 2007; Baran et al., 2000, Schwieler et al., 2007) and following immunotherapy with IFN-α (Raison et al., 2010). In addition, inflammatory prostanoids also seem capable of influencing kynurenic acid production since the COX-1 inhibitor indomethacin cause increased levels of kynurenic acid, while COX-2 inhibitors (e.g., parecoxib and
meloxicam) decrease brain kynurenic acid formation in the rat brain (Schwieler et al., 2005; 2006; 2008).

1.7.2 Neurochemical properties

Kynurenic acid is a neuroactive compound with unique biochemical profile. At low concentrations it antagonizes α7nACh receptors [IC_{50} ≈ 7 µM] (Hilmas et al., 2001), and the strychnine-insensitive glycine site of NMDA receptors [IC_{50} ≈ 8-15 µM] (Birch et al., 1988; Ganong and Cotman, 1986; Kessler et al., 1989; Parsons et al., 1997). At higher concentrations kynurenic acid also antagonizes the glutamate recognition site of NMDA receptors [IC_{50} ≈ 200-500 µM] (Kessler et al., 1989), and with concentrations in the millimolar range it acts as a competitive antagonist at AMPA and kainate receptors (Bertolino et al., 1989; Kessler et al., 1989). In addition, kynurenic acid has been recognized as an endogenous ligand of the G protein-coupled receptor 35 (GPR35), IC_{50} = 39 µM (Wang et al., 2006). This receptor is mainly expressed in the gastrointestinal tract and immune cells, but lower levels have also been detected in various regions of the human brain (Wang et al., 2006). Unpublished data from our laboratory show that the GPR35 receptor is expressed in cultured human and rat astrocytes.

1.8 IMMUNOLOGICAL ASPECTS

The brain was previously considered to be an immune-privileged site due to the absence or low expression of major histocompatibility complex (MHC) molecules within the CNS and the presence of the blood-brain barrier. This idea has, however, been modified and it is now clear that a complex and bidirectional communication exists between the immune system of the brain and that of peripheral organs, and that lymphatic cells infiltrate the CNS under certain conditions (Dantzer et al., 2008). Cytokines serve as the communication molecules of the immune system. They include a vast variety of pleiotropic molecules grouped according to protein family as interleukins, chemokines, interferons, tumor necrosis factors and colony-stimulating factors. Although no longer considered an immune-privileged site, the immune responses of the brain do differ from those in the periphery and there is a notable lack of correlation between the amounts and types of cytokines being released in the CNS and in the periphery under physiological and pathological conditions (Hasegawa et al., 2011; Kawabe et al., 2010; Maier et al., 2005; Trysberg et al., 2000). Moreover, several pro-inflammatory cytokines and their receptors are constitutively expressed in the brain under physiological conditions, and recent data suggests that they influence both synaptic plasticity and behavior.

1.8.1 Brain immune responses

In the brain, glial cells have an important role in mediating immune responses. Glial cells are divided into macroglia and microglia, where the latter type are resident myeloid cells that together with perivascular macrophages constitute the primary active immune defense in the CNS. Microglia have a widespread, non-overlapping
distribution in the brain and spinal cord where they continuously monitor their surroundings for cellular damage and infectious agents. Under normal conditions these cells are quiescent and immune activity is kept to a minimum within the CNS, but once activated they become highly motile cells, capable of phagocytosis, that swiftly mediate a powerful inflammatory response. Microglia mediate innate immune responses via the activation of Toll-like receptors (TLR) that recognize conserved pathogen-associated molecular patterns (PAMPs) on infectious agents (Falsig et al., 2008). Also, astrocytes, macroglial cells abundant in grey matter, express TLRs and are involved in the brain innate immune response (Falsig et al., 2008; Farina et al., 2007). TLR signaling in microglia and astrocytes induces the production of an array of pro-inflammatory chemokines, prostaglandins and cytokines such as IFNs, TNF, IL-1β and IL-6 and promotes cell proliferation (Dantzer et al., 2008; Falsig et al., 2008; Farina et al., 2007; Griffin, 2003). These inflammatory mediators activate neighboring cells and thereby amplify the innate immune response, as well as increase blood-brain permeability and attract circulating immune cells needed for adaptive immune responses. When activated, microglia (and to some extent astrocytes) also upregulate the expression of MHC molecules, enabling them to present antigens to recruited leukocytes and initiate adaptive immune responses. Further, meningeal and perivascular macrophages, situated along the blood-brain barrier, also play a crucial role in mediating the adaptive immune response in the brain by promoting influx of leukocytes across the blood-brain barrier when activated (Polfliet et al., 2001).

The brain also monitors peripheral immune responses and produces sickness-behavior, a motivational state with several similarities to depression that follows the course of an infection and includes symptoms like fatigue, reduced interest in social activities, inability to experience pleasure, increased sensitivity to pain, lowered food intake, changes in sleep patterns and cognitive impairment (Kelley et al., 2003). There are several possible routes by which the brain can respond to peripheral immune stimuli. For instance, afferent neural pathways (e.g., the vagus and glossopharyngeal nerves) can be activated during abdominal or oro-lingual infections (Bluthe et al., 1994; Bret-Dibat et al., 1995; Romeo et al., 2001). Macrophage-like cells resident in the choroid plexus (a capillary bed producing CSF and forming the blood–CSF barrier), circumventricular organs or meninges, as well as brain vascular endothelial cells respond to circulating immune stimuli by producing pro-inflammatory mediators that are released in the brain parenchyma or CSF (Quan et al., 1998; Reyes et al., 1999). Moreover, the blood-brain barrier changes its permeability during infection and circulating cytokines can enter the brain by crossing through volume diffusion or via saturable transporters (see Banks and Erickson, 2010).

### 1.8.2 Cytokines and behavior

Psychiatric symptoms and cognitive impairments are known to co-occur with a number of inflammatory conditions including infectious diseases (e.g., human immunodeficiency virus, hepatitis C viral infection, malaria) and autoimmune disorders (e.g., systemic lupus erythematosus, multiple sclerosis). Several pro-inflammatory cytokines are constitutively expressed in the brain under physiological conditions and high expression of receptors of the pro-inflammatory cytokines IL-1β and IL-6 can be
found in the hippocampus, cortex and hypothalamus (Parnet et al., 1994; Schobitz et al., 1992, for review see Tonelli and Postolache, 2005). Recent studies analyzing the CSF content of cytokines in psychiatric disorders have shown increased levels of IL-1β in first episode patients with schizophrenia (Söderlund et al., 2009) and elevated levels of IL-6, which correlate to symptom severity, in suicide attempters (Lindqvist et al., 2009). Further, pro-inflammatory cytokine therapy as treatment for autoimmune diseases, cancers or viral infections is associated with the development of psychiatric symptoms that occur approximately after one month of treatment and terminate with cessation of the therapy. Accordingly, IFN-α treatment for cancer or hepatitis C is often associated with symptoms of depression, but also with anxiety, delirium, cognitive impairments, irritability, mixed states, hypomania and mania (Capuron et al., 2002; Capuron and Miller, 2004; Constant et al., 2005; Giunta et al., 2007; Raison et al., 2005). Similarly, IFN-β treatment in multiple sclerosis is associated with depression (Galeazzi et al., 2005) and in some cases phobic, aggressive, psychotic and manic symptoms (Fragoso et al., 2010). IFNs are potent stimulators of other pro-inflammatory cytokines both in the periphery and the brain and, for instance, INF-α treatment is known to increase the CSF levels of IL-6 in patients treated for hepatitis C (Raison et al., 2009). They also potently induce IDO, one of the rate-limiting enzymes in the synthesis of kynurenic acid from tryptophan (see section 1.7.1.1). Induction of IDO causes increased levels of neuroactive metabolites such as kynurenic acid and might also decrease levels of serotonin by reducing the bioavailability of its precursor tryptophan. Alterations in the synthesis of neuroactive metabolites and neurotransmitters caused by the induction of IDO could thus be one way in which pro-inflammatory cytokines mediate changes in mood and behavior. Moreover, microglia, astrocytes and neurons express various cytokines and cytokine receptors and emerging data suggest that some cytokines function as modulators of neurotransmission (see section 1.8.4).

In animals, sickness behavior including altered sleep pattern, cognitive deficits, decreased motor activity, social withdrawal, anxiety-like behavior, anhedonia, and reduced intake of food and water can be induced by peripheral or central administration of lipopolysaccharide (LPS) or pro-inflammatory cytokines such as IL-1β or TNF (Dantzer, 2001). Interestingly, there is a temporal dissociation between the “sickness” behaviors (i.e., neurovegetative symptoms) and the depressive-like symptoms and cognitive deficits in response to immune stimuli, where sickness behavior occurs earlier and depressive-like symptoms and cognitive deficits develop later. In animals subjected to LPS, sickness behavior including fatigue develops rapidly and peaks between 2-6 hours after treatment. After 6 hours, the sickness symptoms gradually subside and depressive-like behavior and cognitive deficits emerge (for review see Capuron and Miller, 2011). Peripheral administration of LPS, as well as acute stress, is known to promote local production of IL-1β and other pro-inflammatory cytokines within the brain (Bay-Richter et al., 2011; Laye et al., 1994; Quan et al., 1999; Sugama et al., 2011). Several studies have highlighted the role of centrally-produced IL-1β in the generation of depressive-like behavior. Thus, IL-1β produced locally within the brain after peripheral LPS administration continues to rise after sickness behavior has subsided and depressive-like symptoms are present (Bay-Richter et al., 2011). Further,
the intracerebroventricular (i.c.v.) administration of IL-1ra reverse the depression-like behavior induced by peripheral LPS (Konsman et al., 2008). Mood dysregulation has classically been linked to various disturbances in monoamine neurotransmitter systems and interestingly, subchronic elevation of IL-1β mediates profound alterations in monoamine metabolism including decreased norepinephrine levels in several limbic regions, increased dopamine levels in the amygdala (~60%) and midbrain (~80%), and decreased dopamine levels in the frontal cortex (Song et al., 2006; 2008).

1.8.3 Neuron-glia communication

Our knowledge on the functional role of glial cells has greatly improved over the last decades. It is now known that these cells are far more than the mere “glue” holding neurons in place, and that they play a key role in brain physiology and pathology. For instance, glial cells supply nutrients and oxygen to neurons, control electrolyte homeostasis, form myelin, produce neuromodulators and trophic factors, regulate neurotransmitter release, modulate synaptic plasticity, mediate immune responses and regulate neuronal migration and synapse formation during brain development.

In being part of the same system, microglia, astrocytes and neurons need matching communication molecules and receptors. Astrocytes and microglia express a number of neurotransmitter receptors (e.g., glutamate, norepinephrine, serotonin, histamine, acetylcholine and GABA receptors) and release several neurotransmitters (also known as gliotransmitters) including glutamate, aspartate and ATP (for review see Hansson and Rönnback, 2003). In addition, they also release neuromodulators such as D-serine, kynurenic acid, nitric oxide, free radicals, cytokines and chemokines. As part of the tripartite synapse (see figure 2) astrocytes are intimately associated with glutamatergic pre- and postsynaptic elements where they bidirectionally influence neurotransmission by regulating glutamate uptake and by releasing neurotransmitters and neuromodulators (for review see Perea et al., 2009). D-serine released by astrocytes has for instance been demonstrated to play a pivotal role in the production of NMDA-dependent LTP (Henneberger et al., 2010; Yang et al., 2003). Further, the stimulation of single astrocytes produces lasting potentiation of synapses in both the hypothalamus and hippocampus (Gordon et al., 2009; Perea and Araque, 2007), indicating that astrocytes are able to modulate synaptic plasticity.

![Figure 2](image)

Figure 2. The tripartite synapse.

other via gap junctions forming a network of connected cells that can propagate intracellular $\text{Ca}^{2+}$ waves. Recent data show that such $\text{Ca}^{2+}$ waves are triggered by neurotransmitter stimulation, which in turn mediates the release of gliotransmitters and neuromodulators, as illustrated in figure 2 (for reviews see Achour and Pascual, 2010; Haydon, 2001; Malarkey and Parpura, 2008). In light of this, astrocytes might be capable of monitoring and orchestrating synaptic strength and neurotransmission over larger brain regions. Whether or not astrocytes can function as interconnected networks has been questioned by an in vivo study demonstrating that astrocytes in the visual cortex respond to visual stimuli together with their neighboring neurons in a single-cell restricted manner (Schummers et al., 2008). Even though astrocyte function needs to be more thoroughly investigated, compelling in vitro and in vivo data suggest that they play an active role in informational processing in the brain.

### 1.8.4 Cytokines as neuromodulators

IL-1β and its receptors are widely distributed throughout the rodent brain with high densities in the hippocampus (Ban, 1994; Farrar et al., 1987; Lechan et al., 1990; Parnet et al., 1994). Postmortem studies have confirmed that IL-1β, IL-1R and IL-1ra are also expressed in the cortex, hippocampus and hypothalamus in the human brain (Breder et al., 1988; Cacabelos et al., 1994; Hammond et al., 1999; Rao et al., 2010; Toyooka et al., 2003; Yasuhara et al., 1997). There are two types of IL-1Rs, the IL-1RI and the IL-1RII, where only the type I receptor mediates intracellular signaling. It was recently discovered that IL-1RI receptors are co-localized and bound to NMDA receptors in hippocampal synapses (Gardoni et al., 2011). Stimulation with IL-1β causes a dose-dependent increase in NMDA receptor mediated $\text{Ca}^{2+}$ influx (with a maximal effect of 45%) by increasing phosphorylation of the NMDA receptor subunits NR2A and NR2B (Viviani et al., 2003). In addition, IL-1β can influence glutamate signaling by altering the expression and phosphorylation of AMPA receptors (Lai et al., 2006). In further support of a functional role for IL-1β in synaptic plasticity, the endogenous production of IL-1β has been found to contribute to in vivo and in vitro LTP in rats (Schneider et al., 1998). Several behavioral studies support this finding as various hippocampal-dependent memory deficits are observed, both in animals with decreased IL-1 signaling (IL-1R knockout animals or local infusion of IL-1ra) as well as in transgenic animals overexpressing IL-1β (Avital et al., 2003; Hein et al., 2011; Yirmiya et al., 2002). Thus, local administration of the pro-inflammatory cytokine IL-1β has been shown to modulate glutamate transmission and synaptic plasticity, cause profound alterations in brain monoamine levels, and produce altered behavior in rodents. Interestingly, another pro-inflammatory cytokine, IL-6, is also endogenously produced during in vivo and in vitro LTP, but in contrast to IL-1β, it has been shown to aid the annihilation of LTP (Balschun et al., 2004). Consistent with this concept, blockade of IL-6 causes improvement in long-term memory (Balschun et al., 2004) and IL-6 knockout mice show enhanced memory performance in the eight-arm radial maze (Braida et al., 2004).
2 SPECIFIC AIMS OF THE STUDY

- To examine the effects of elevated brain kynurenic acid on amphetamine-induced dopamine transmission.

- To examine the effects of elevated brain kynurenic acid on spontaneous and amphetamine-induced locomotor activity.

- To analyze the cerebrospinal fluid content of kynurenic acid and cytokines in patients with bipolar disorder and in healthy volunteers.

- To evaluate the relationship between the CSF levels of kynurenic acid or cytokines and clinical symptoms in patients with bipolar disorder.
3 MATERIALS AND METHODS

3.1 ANIMALS AND ETHICAL ASPECTS

In order to investigate the effect of brain kynurenic acid on dopaminergic transmission by electrophysiology and microdialysis (paper I) male Sprague Dawley rats were used (Scanbur BK, Sollentuna, Sweden). The rats were housed in groups of 3-4 and weighed between 180 g (on the day of surgery) and 250 g (on the day of experiment). For behavioral experiments (paper II) male C57BL/6 mice (Charles River, Sulzfeld, Germany) were used. Mice, 13-15 weeks of age, were housed in groups of 2-6 and handled daily at least 10 days prior to the experiments to reduce handling stress. All animals were checked daily and kept in a room with constant humidity (40-60%), temperature (25°C) and 12-h light/dark cycle (lights on 06.00 hours). Food and water was provided ad libitum. All experiments were approved by and performed in accordance with the guidelines of the Ethical Committee of Northern Stockholm, Sweden. All efforts were made to minimize the number of animals used and their suffering.

3.2 DRUGS AND CHEMICALS

Drug weights were calculated as the salt. d-amphetamine sulphate (Apoteksbolaget, Göteborg, Sweden) and L-kynurenine (Sigma-Aldrich, St Louis, MO, USA) were dissolved in deionized water. L-kynurenine was pH adjusted to 5.7 with NaHCO$_3$ (paper I) or to 8.1-9 with NaOH (paper II) before injection. Apomorphine was dissolved in 0.1% ascorbic acid solution. Chloral hydrate, general anesthetic drug, was dissolved in deionized water (Merck KGaA, Darmstadt, Germany). Isoflurane was used for general anesthesia (Abbott Scandinavia AB, Solna, Sweden) and bupivacaine hydrochloride was used for local anesthesia (Astra Zeneca, Sweden). All other chemicals used were of the highest commercial quality.

3.3 ELEVATION OF ENDOGENOUS KYNURENIC ACID

As kynurenic acid does not readily cross the blood-brain barrier, elevation of endogenous levels of brain kynurenic acid was achieved by systemic administration of its blood-brain barrier crossing immediate precursor L-kynurenine. For acute elevation, Sprague Dawley rats (paper I) were pretreated with a single dose of L-kynurenine (5 mg/kg) subcutaneously (s.c.) 60 min prior to experiments, while C57BL/6 mice (paper II) received an intraperitoneal (i.p.) dose of 10 mg/kg L-kynurenine 60 min prior to experiments. To elevate endogenous brain kynurenic acid levels subchronically, Sprague Dawley rats (paper I and PET study) had two surgically implanted s.c. osmotic minipumps (2ML1 Alzet, Cupertino, CA, USA) delivering L-kynurenine at a continuous flow rate of 10 ml/h for 6 days. The dose was adjusted to 90 mg/kg/day, at the day of implantation. The solutions were passed through a sterile filter (Acrodisc Syringe Filter 13 mm with 0.2 mm Supor1 membrane) before the filling of
pumps. C57BL/6 mice (paper II) were pretreated subchronically with 100 mg/kg L-kynurenine i.p. injections every 12 hours for 6 days.

### 3.4 ANESTHESIA AND SURGERY FOR SUBCHRONIC ELEVATION OF ENDOGENOUS KYNURENIC ACID (PAPER I AND PET STUDY)

For induction of anesthesia, rats were placed in a Plexiglas chamber filled continuously with 4.8% isoflurane in air using a vaporizer (Univentor 400 Anesthesia Unit; Univentor Ltd, Zejtun, Malta). Animals were then placed on a heating pad maintaining body temperature at 37 °C throughout the surgery (Temperature Control Unit, HB 101/2, AgnTho’s AB, Lidingö, Sweden) and anesthesia was maintained with a continuous flow of 2.4% isoflurane delivered via a nose cone. For post-operative analgesia, 0.5 ml bupivacaine (5 mg/ml) was administered s.c. The minipumps were inserted through an incision in the neck and placed s.c. on the back. After surgery rats were housed in single cages for observation 24 h before being reunited in groups of 3-4. Blood collected from the lateral tail vein during surgery was compared with blood collected at day 6 and verified a 3.2-fold increase of blood kynurenic acid concentration by the subchronic L-kynurenine administration.

### 3.5 IN VIVO ELECTROPHYSIOLOGY

#### 3.5.1 Anesthesia and surgery

Rats were administered an i.p. injection of 8% chloral hydrate (400 mg/kg) and left in a quiet environment for 10-15 minutes for induction of anesthesia. If the level of anesthesia was unsatisfactory an additional 0.5 mL of 8% chloral hydrate was administered i.p. The rat was placed on a heating pad maintaining the body temperature at 37 °C and mounted onto the ear bars of a stereotaxic frame (David Kopf Instruments, Tujunga, CA, USA). The head was fixed in a horizontal plane by securing the nose in a clamp at the front of the frame. A cannula was inserted into a lateral tail vein for intravenous (i.v.) administration of drugs. The level of anesthesia was determined by the animal’s reaction to hind paw pinching and its breathing pattern. A level of surgical anesthesia was maintained during the experiment by additional i.v. administrations of 8% chloral hydrate. The skull surface was exposed by a medial incision from the nose base to the base of the skull, and 0.1 ml of bupivacaine (5 mg/ml) was allowed to soak for 5 minutes for additional local anesthesia before removal of the periosteum. A 3 mm burr hole was drilled with its center approximately 3.0 mm anterior to lambda and 0.7 mm to the right lateral side of the skull. The dura was carefully removed using a curved cannula.

#### 3.5.2 Recording electrode

Single barrel recording electrodes were prepared from glass capillaries with 1.16 mm inner diameter (Harvard Apparatus). The capillaries were pulled in a vertical electrode puller (Narishige, Japan) set at 14.5 amperes, and filled with 0.5 M sodium acetate

---

38
saturated with Pontamine Sky Blue. The tip of the electrode was broken under a microscope forming a diameter of approximately 1-2 µm. The \textit{in vitro} impedance was between 6-8 MΩ measured at 135 Hz in 0.9% saline.

### 3.5.3 Extracellular single cell recording

The glass microelectrode was mounted onto a hydraulic microdrive (David Kopf Instruments, Tujunga, CA, USA) fastened to the stereotaxic frame. The electrode was adjusted to the coordinates of the VTA according to the atlas of Paxinos and Watson (Paxinos and Watson, 1998), approximately 3 mm anterior to lambda and 0.7 mm to the right lateral side, and vertically lowered into the brain to a depth of 7 mm. From this point the electrode was gently lowered using only the hydraulic microdrive. All dopamine neurons were found 7.5–8.5 mm from the brain surface. Signals identified as dopamine neuron single unit potentials were passed through a high input impedance amplifier and filters. The impulses were discriminated from background noise and fed into a computer while being simultaneously monitored on an oscilloscope (Gould 500 digital storage oscilloscope, Essex, UK), audio monitor (Grass AM8 Audio monitor, Grass Medical Instruments, Quincy, MASS, USA) and a strip chart recorder (Gould Instruments, Ohio, USA). Following the experiment, rats were euthanized by an overdose of 8% chloral hydrate i.v. The recording sites of the electrophysiological experiments included in this thesis were not histologically verified as the brain tissues were analyzed for kynurenic acid content. Instead the dopaminergic neurons were identified on their typical electrophysiological characteristics (see section 3.5.4). When experiments allowed, a single dose of the dopamine agonist apomorphine (100 mg/kg i.v.) was administered at the end of the experiment to confirm that the recording had been made from a dopamine neuron.

### 3.5.4 Electrophysiological characteristics of dopaminergic neurons

Dopamine neurons have three states of activity: nonfiring (due to inactivation or hyperexcitation leading to a depolarization blocked state), single spike firing or burst firing (Grace and Bunney, 1984b). The electrophysiological characteristics of dopamine neurons include \textit{i}) a biphasic (positive-negative) or triphasic (positive-negative-positive) waveform, often with a prominent inflection in the initial phase, \textit{ii}) a duration of the action potential of >2 msec giving rise to a characteristic low pitched sound on the audiomonitor, and \textit{iii}) single spike firing or burst firing in a firing frequency between 1-10 Hz (Grace and Bunney, 1984a; 1984b; Wang, 1981). Spontaneous single spike firing is characterized by an irregular spike pattern with steady firing rate. When in burst mode, dopamine cells typically fire three to six spikes in bursts with progressively decreasing amplitude and increased duration. Increase in firing rate is associated with simultaneous increased burst firing, and it is hypothesized that single spike dopamine neurons switch to burst firing mode in response to an increased demand of neurotransmission given the massive increase in dopamine release associated with the latter state (Gonon, 1988; Grace and Bunney, 1984a). In anesthetized animals about 50% of the dopamine neurons are spontaneously burst firing whereas over 90% of dopamine neurons are bursting in freely moving rats (Freeman...
Materials and Methods

and Bunney, 1987; Grace and Bunney, 1984a). Lack of sensory and movement induced stimulation as well as influence by anesthetics could be the cause for this divergence.

3.5.5 Drug administration

Drugs were administered i.v. via the lateral tail vein. Spontaneous firing activity was recorded approximately 3 minutes before drugs were administered. The effect of incremental doses of d-amphetamine (0.2-25.6 mg/kg) on firing and percent burst firing was recorded and compared with predrug levels. Since d-amphetamine has an inhibitory action on dopaminergic firing activity the dose acquired for effectively silencing a dopamine neuron was also investigated. Cells were considered silenced when no firing occurred during at least 30 consecutive seconds following d-amphetamine administration.

3.5.6 Influence of anesthesia

The influence of chloral hydrate anesthesia cannot be ruled out as a confounding factor on neural activity during electrophysiology experiments. The firing pattern of dopaminergic neurons in anesthetized animals is known to have somewhat different characteristics, with for instance lower burst frequencies, then in awake and freely moving rats (see section 3.5.4). Electrophysiology data from sedated animals might therefore not always be directly translational to behavior seen in awake and freely moving rats. The active metabolite of chloral hydrate, trichloroethanol, impairs NMDA receptor function (Peoples and Weight, 1998; Scheibler et al., 1999) and is possibly responsible for the depressant effects by chloral hydrate on the CNS (Breimer, 1977). Chloral hydrate does not alter basal or cocaine-evoked dopamine levels in rat striatum, however, it has been shown to decrease extracellular levels of glutamate (Kreuter et al., 2004). These effects on glutamatergic transmission might interfere with some of the effects caused by elevated brain kynurenic acid. However, since the results from L-kynurenine pretreated animals in this thesis are consistently compared to a control group of rats with the same anesthetic and experimental protocols, the effects observed should be attributed to L-kynurenine pretreatment and the molecular effects associated with it.

3.5.7 Data analysis

Spike distribution was analyzed online utilizing a Spike II software program (Cambridge Electronic design, Cambridge, England) on a Hewlett-Packard Compaq computer. The software was designed to sample and analyze the intervals of an arbitrary number of transistor-transistor logic (TTL) pulses (corresponding to spikes passing through the discriminating filter) with a time resolution of one msec. An interspike interval (ISI) was defined as the time in msec between the rising edges of two consecutive TTL pulses. In order to avoid artifacts in the sampling procedure, the spike analyzer was set to ignore time-intervals below 20 msec. Burst onset was determined as an ISI shorter than 80 msec and its termination by the next interval
longer than 160 msec (Grace and Bunney, 1984a; 1984b). Cells were considered to be bursting if at least one ISI of 100 recorded spikes was shorter than 80 msec. The cell firing and percent burst firing activity was generally calculated over successive periods of 100–300 spikes. At very low firing frequencies (less than 2 Hz) following amphetamine administration, cell firing and percent burst firing activity was occasionally calculated over consecutive periods of less than 100 spikes.

### 3.6 MICRODIALYSIS

Microdialysis was used to measure the extracellular levels of dopamine within the nucleus accumbens of freely moving rats to examine the effects of elevated brain kynurenic acid on amphetamine-evoked dopamine release. To reduce effects of inter-individual variations due to differences in probe recovery the dialysate concentrations were transformed to percent of baseline before statistical analysis.

#### 3.6.1 Anesthesia and surgery

Rats were anesthetized with isoflurane as described in section 3.4 and mounted onto the ear bars of a conventional stereotaxic frame (David Kopf Instruments, Tujunga, CA, USA). The skull was exposed and two shallow holes were drilled for insertion of anchor screws. Another hole was drilled above the nucleus accumbens. The incisor bar was adjusted so that the skull was set in a horizontal flat plane and following careful removal of the dura, a guide cannula (MAB 9.IC, AgnTho’s AB) was directed to the region of nucleus accumbens. Stereotaxic coordinates for the implantation of the guide cannula with reference to bregma and brain surface, respectively, were: AP +1.6, ML 1.4, DV +6.2, placing the tip of the guide cannula 2 mm above the vertical target for the final position of the microdialysis probe. The guide cannula was fixed with stainless-steel screws and dental cement (Dentalon1, AgnTho’s AB). During surgery, 0.5 mL bupivacaine (5 mg/mL) was administered s.c. to provide post-surgical analgesia. Rats were single caged for 24 h with free access to food and water. On the following day, microdialysis was performed on unanesthetized freely moving rats.

#### 3.6.2 Experimental protocol and analysis of dopamine

The guide was removed and a microdialysis probe (MAB 9.14.2, polyether sulphone, 2 mm dialysing length, 0.56 mm diameter and a 15 kDa cut-off membrane; AgnTho’s AB) was inserted into the cannula. Rats were connected to microdialysis swivels and the probes were perfused with perfusion fluid CNS (CMA Microdialysis AB, Solna, Sweden) delivered via polyethylene tubing from an infusion pump (Univentor 864, Univentor Ltd) at a flow rate of 2 ml/min. Thirty-minute fractions were collected using a microfraction collector (Univentor 820, Univentor Ltd) and manually injected (Rheodyne, Cotati, CA, USA) into a high-performance liquid chromatography (HPLC) system. Baseline was usually obtained after 2–3 h and consisted of three consecutive samples with a maximal variation of 10%. Results for subsequent samples were calculated as percentages of this average basal release. Rats were then administered 2
mg/kg d-amphetamine i.p. and dopamine concentration was measured for 180 min.

Separation of dopamine was achieved by reversed-phase liquid chromatography using a 55 mM sodium acetate buffer (pH 4.1, 10% methanol) with 0.8 mM octanesulfonic acid and 0.01 mM Na₂-ethylenediaminetetra-acetate (EDTA). The mobile phase was delivered by an HPLC pump (Bischoff Chromatography, Leonberg, Germany) through a ReproSil-Pur C18 column (4x150 mm, Dr Maisch GmbH, Ammerbuch, Germany) at a rate of 0.8 mL/min. Following separation, the analyte was first passed through a guard cell with an oxidizing potential of 50 mV. Samples were then quantified by sequential oxidation and reduction in a high-sensitivity analytical cell (ESA 5011; ESA Inc., Chelmsford, MA, USA) controlled by a potentiostat (Coulochem III; ESA Inc.) with an applied potential of -200 mV for detection of DA. The signals from the detector were transferred to a computer for analysis (Datalys Azur, Grenoble, France). The retention time of dopamine was approximately 8 min.

3.6.3 Histology

Following the experiment, animals were euthanized by decapitation and the brains were carefully removed and fixated by submersion in 10% formalin in neutral buffer for at least 5 days. Serial coronal sections (50 mm) were made using a cryostat (Slee Medical GmbH, Mainz, Germany), and the microdialysis probe placement was verified with reference to the stereotaxic atlas of Paxinos and Watson (Paxinos and Watson, 1998). No distinction could be made between the core and the shell of the nucleus accumbens. Data are reported only from animals where probe membranes were correctly positioned in the nucleus accumbens.

3.7 POSITRON EMISSION TOMOGRAPHY

In order to examine the effects of subchronic elevation of brain kynurenic acid on dopamine transmission, the three-dimensional imaging technique positron emission tomography (PET) was used to study dopamine release in striatum following amphetamine administration in anesthetized rats.

Male Sprague Dawley rats were anesthetized with isoflurane, controlled by an E-Z anesthesia vaporizer (5% initially and then 1.5% to maintain anesthesia, blended with 7:3 air/O₂ and delivered through a Microflex non-rebreather mask from Euthanex Corporation, Palmer, PA). Rats were then placed on a heating pad (37°C) on the camera bed approximately 15 min before injection of tracer. The PET tracer was administered by bolus injection via the lateral tail vein (maximum volume of 1 mL). PET data were acquired in fully three-dimensional (3-D) mode using a microPET Focus 120 camera (CTI Concorde Microsystems). Data were processed using MicroPET Manager and Inveon Research Workplace (Siemens Medical Solutions). Images were reconstructed by standard 2-D filtered back projection using a ramp filter. The matrix size of the reconstructed images was 128x128x95 with a spatial resolution of 1.3 mm. Data were corrected for randoms, dead time and decay. Amounts of [¹¹C]Raclopride ([¹¹C]RAC) injected ranged from 9.3 to 13.1 MBq. PET scanning
started after tracer injection, data acquisition time was 60 min. [¹¹C]RAC was obtained from batches made for clinical PET and had passed standard quality controls. A dose of 2 mg/kg amphetamine (or sterile saline) was administered via injection in the tail vein 10 minutes after the start of data acquisition.

To determine the occupancy of dopamine D₂ receptors in the rat striatum, regions of interest (ROIs) were placed in the left and right striatum as well as cerebellum (reference region). Radioactivity concentrations (Becquerel’s per milliliter) were calculated automatically by calibrating against a phantom with a known concentration of radioactivity. Assuming a tissue density of 1 g/mL, the radioactivity concentrations were divided by the administered activity to obtain a ROI-derived percent injected dose per gram of tissue (%ID/g). When the PET data acquisition was finished, animals were euthanized by cervical dislocation while still under anesthesia.

### 3.8 OPEN FIELD TEST

Spontaneous and amphetamine-induced locomotor activity was studied in an open field arena to evaluate the physiological significance of elevated brain kynurenic acid. To reduce variation in the results all tests were carried out by one and the same person and all animals were handled daily at least 10 days before the experiment to avoid handling stress.

#### 3.8.1 Apparatus

Locomotor activity was recorded by placing the mouse in a square Plexiglas box (50 x 50 x 21.6 cm) within a sound-dampened and solid chamber (ADITECH, Fjärås, Sweden). Photocells sensitive to infrared light formed a two-layer grid over the open field arena (16 cells per row on each side, 3.1 cm apart). Each photocell interruption was counted and registered by a computer. Horizontal activity was measured as the total number of beam breaks in the lower photocell layer, peripheral activity as the total number of beam breaks along a wall in the lower layer, forward locomotion as when the animal makes a real movement by measuring the total count of new beam breaks in the lower layer, rearing activity as when the animal stand on its hind legs by counting the beam breaks in the upper layer, and corner time as the accumulated time in seconds that the animal spend in the corners of the test box.

#### 3.8.2 Experimental protocol

The test consisted of three 60-minute habituation sessions taking place with 24 hours apart. After the third habituation animals were immediately injected with either d-amphetamine (5mg/kg, i.p.) or saline and locomotor activity was recorded for an additional 90 minutes. All tests were performed in the dark during the animals’ light cycle, between 6 a.m. and 6 p.m., and the mice were transferred to the experimental room ~30 minutes prior to each session. After the final test, animals were swiftly sedated with isoflurane in a Plexiglas chamber and sacrificed by cervical dislocation.
3.9 SAMPLING OF HUMAN CEREBROSPINAL FLUID

3.9.1 Ethical aspects

Studies included in this thesis were approved by the Regional Ethical Committees of the University Hospital in Linköping, the Karolinska Institutet and the Medical Faculty of Uppsala University, Sweden. The work described was carried out in accordance with “The code of ethics of the World Medical Association” (Declaration of Helsinki) for experiments including humans: http://www.wma.net/en/30publications/10policies/b3/.

3.9.2 Subjects

Bipolar disorder patients meeting the Diagnostic and Statistical Manual of Mental Disorders (DSM)-IV criteria for bipolar disorder were recruited from December 2005 to April 2008 from a long-term follow-up program (St. Göran bipolar project) at a bipolar outpatient unit at the Northern Stockholm psychiatric clinic, Stockholm, Sweden. At the time of enrollment, this clinic served an area with a total population of 316,400 persons over 18 years of age, covering a wide range of demographic characteristics including both areas with a high proportion of native-born Swedes and high income as well as ethnically diverse areas with high deprivation indices. All patients in this area showing symptoms of mania, hypomania or other signs of bipolar disorder received initial care at this clinic.

The clinical diagnosis for bipolar disorder was the Affective Disorder Evaluation (ADE), which was previously used in the systematic treatment enhancement program for bipolar disorder (STEP-BD) project (Sachs et al., 2003). With the permission of the originator Gary S. Sachs, the ADE was translated and modified to suit Swedish conditions. The ADE starts with a social anamnesis, followed by the affective module of the Structured Clinical Interview for DSM-IV (Spitzer et al., 1992). The number of lifetime affective episodes and their characteristics were documented. Other modules assessed alcohol and drug abuse, violent behavior, childhood history, family history, treatment history, reproductive history and somatic illnesses. Interpersonal violence was defined as a violent act or serious physical threat to another person. Suicide attempt was defined as a deliberate and serious self injury, including intoxication with medication, with the intent to die or seriously harm oneself. In addition to the ADE, the Mini International Neuropsychiatry Interview (M.I.N.I.; Sheehan et al., 1998) was completed at baseline to screen for psychiatric diagnoses other than bipolar disorder. To screen for alcohol and substance abuse, patients completed two self report questionnaires: the Alcohol Use Disorders Identification Test (AUDIT; Saunders et al., 1993) and the Drug Use Disorders Identification Test (DUDIT; Berman et al., 2005).

The ADE and M.I.N.I. interviews were conducted by board certified psychiatrists working at the bipolar outpatient unit, or by residents in psychiatry completing their psychiatric training at this unit. The assessments were based on patient records and interviews with next of kin when feasible. To minimize interrater variability, the collected patient data was presented at a diagnostic case conference, where a consensus panel of experienced board certified psychiatrists specialized in bipolar disorder made the final diagnostic decision. Consecutive new outpatients referred for treatment and
continuing patients at the bipolar outpatient unit were invited to participate, provided that they were at least 18 years old and met the DSM-IV criteria for any bipolar disorder, i.e., type I, II, NOS, cyclothymia, or schizoaffective syndrome manic type. The invited participants obtained detailed verbal and written or oral information about the study and all participants gave their written informed consent. Patients were excluded from the study if they were unable to complete the standard clinical assessment or were incapable of providing informed consent.

The studies presented in this thesis include patients diagnosed with bipolar disorder type I (paper III, IV and V) and II (paper III and V). All CSF samples were collected when the patients were symptom free and in a stable euthymic mood, as judged by a physician. For ethical reasons, the patients continued to take their prescribed medication. In paper III, all but 2 patients received on-going medication at the time of sampling. The most commonly used treatments were: lithium (66%), lamotrigine (23%), quetiapine (16%), mirtazapine (13%), propiomazine (13%) and valproic acid (10%). In paper IV, all patients received on-going medication at the time of sampling. The most commonly used treatments were: lithium (67%), lamotrigine (22%), propiomazine (15%), quetiapine (13%), olanzapine (11%) and mirtazapine (11%). In paper V, all but two patients received pharmacological treatment at the time of sampling. Patients were taking the following medications: lithium (57%), lamotrigine (27%), quetiapine (20%), valproic acid (20%), mirtazapine (13%) and propiomazine (13%).

Healthy male volunteers were recruited among medical students, hospital staff members and their relatives in Linköping, Sweden. They all underwent a medical check-up including laboratory tests (electrolytes, blood, thyroid, kidney and liver) and a physical examination. The volunteers were medication free for at least 1 month and free from any form of substance abuse. Coffee consumption and smoking was allowed. The volunteers underwent a semistuctured interview using the Structured Clinical Interview for DSM-IV Axis I disorders (SCID-I; First et al., 1997a). The interview was directed toward affective disorders, anxiety disorders and drug abuse. The volunteers also completed the Structured Clinical Interview for DSM-IV Axis II Personality Disorders (SCID-II) questionnaire for personality disorders (First et al., 1997b). All volunteers included in the study were considered healthy by the psychiatrist performing the examinations. They showed no signs of psychiatric or somatic illness and had no laboratory test results outside of the standardized reference ranges. None of the volunteers had a family history of major psychosis or suicide in first- or second-degree relatives. All healthy volunteers received verbal and written information and gave their written informed consent. Participants were excluded if they were unable to complete the standard clinical assessment or were incapable of providing informed consent.

3.9.3 Lumbar puncture

Lumbar puncture was performed on all subjects between 8 am and 11 am after a night of fasting and bed rest. A disposable needle (BD Whitacre Needle, 0.7 x 90 mm) was inserted at the L4–L5 level. In total, 12 mL CSF was collected, inverted to avoid gradient effects, divided into aliquots and frozen at −70°C until assayed.
3.10 ANALYSIS OF KYNURENIC ACID

3.10.1 Sample preparation

Following euthanasia in electrophysiology and behavioral experiments animals were immediately decapitated and the brain (paper I and II) and blood (paper I) were collected and stored in -20 °C. In microdialysis experiments, rats were euthanized by decapitation and blood was collected and stored in -20 °C.

In paper I, the rat brains were weighed and sonicated with equal volumes (assuming a brain density of 1 g/cm³) of homogenization medium (0.4 M perchloric acid solution with 0.1% Na₂S₂O₅ and 0.05% EDTA). Samples were centrifuged at 20’000 g for 5 min. Supernatants were diluted x1.13 with 70% perchloric acid and centrifuged twice at 20’000 g for 5 min. Supernatants were stored in -20 °C for subsequent analysis of kynurenic acid. Blood samples (paper I) were thawed and centrifuged at 20’000 g for 5 min. Supernatants were diluted with equal amounts of homogenization medium (0.4 M perchloric acid solution with 0.1% Na₂S₂O₅ and 0.05% EDTA) and centrifuged again at 20’000 g for 5 min. Supernatants were diluted x1.14 with 70% perchloric acid and centrifuged twice at 20’000 g for 5 min. Supernatants were stored in -20 °C for subsequent analysis of kynurenic acid.

In paper IV, mice brains were weighed and homogenized with 7 times the volume (assuming a brain density of 1 g/cm³) of homogenization medium (0.66M perchloric acid solution with 0.1% Na₂S₂O₅ and 0.05% EDTA) using an electrical disperser (T10 basic Ultra-Turrax, IKA Werke GmbH & Co. KG, Staufen, Germany). Samples were centrifuged at 12’500 g for 5 min, and supernatants diluted x1.1 with 70% perchloric acid. Samples were centrifuged again at 20’000 g for 5 min and supernatants stored in -20 °C for subsequent analysis of kynurenic acid.

Before analysis with HPLC, all samples were thawed and centrifuged at 20’000 g for 5 min.

3.10.2 High performance liquid chromatography

For analysis of kynurenic acid, an isocratic reversed-phase HPLC system with fluorescence detection was used. The system included a dual-piston high-liquid delivery pump (Bischoff, Leonberg, Germany), a ReproSil-Pur C18 column (4×150 mm, Dr. Maisch GmbH, Ammerbuch, Germany) and a fluorescence detector (Jasco Ltd, Hachioji city, Japan) with an excitation wavelength of 344 nm and an emission wavelength of 398 nm (18 nm bandwidth). A mobile phase of 50 mM sodium acetate (pH 6.2, adjusted with acetic acid) and 7.0% acetonitrile was pumped through the reversed-phase column at a flow rate of 0.5 mL/min. Samples of 30-50 µL were manually injected together with mobile phase in a total injection volume of 100 µL (ECOM, Prague, Czech Republic). Zinc acetate (0.5 M, not pH adjusted) was delivered after the column by a peristaltic pump (P-500, Pharmacia, Uppsala, Sweden) at a flow rate of 0.10 mL/hr. Signals from the fluorescence detector were transferred to a computer for analysis with Datalys Azur (Grenoble, France, version 4.6.0.0; http://datalys.net). The retention time of kynurenic acid was about 7–8 min. Initially, the sensitivity of the system was verified by analysis of a standard mixture of kynurenic
acid with concentrations from 0.3125 to 30 nM, which resulted in a linear standard plot. To verify the reliability of this method, some samples were analyzed in duplicate, and the mean intraindividual variation was below 5%.

3.11 ANALYSIS OF CYTOKINES AND CHEMOKINES

Cerebrospinal fluid samples were stored in -70°C until assayed. A sandwich immunoassay-based protein array multiplex system was used (Invitrogen AB) with a guaranteed lowest detection limit of 1 pg/mL for each cytokine to quantify; IL-1β, IL-2, IL-4, IL-5, IL-6, IL-8, IL-10, granulocyte-macrophage colony-stimulating factor (GM-CSF), IFN-γ and TNF. Samples were incubated with specific antibody coated beads. Thereafter, incubations with biotin conjugated detection antibodies and streptavidin-phycoerythrin were conducted. Standard curves (Biosource International) ranging from 0.38 pg/mL to 1’025 pg/mL of the respective cytokine were used for quantification. We used a Luminex reader (Luminex Corporation) to simultaneously quantify the concentrations of the cytokines.

3.12 ANALYSIS OF HOMOVANILLIC ACID

Determination of CSF HVA was performed by means of high-performance liquid chromatography with electrochemical detection. A Pharmacia-LKB 2150 solvent delivery system (Pharmacia Biosystems, Sollentuna, Sweden) was used together with a Rheodyne 7125 injector, an electrochemical detector with a glassy carbon working electrode (model LC-4B, Bioanalytical Systems, West Lafayette, In) and a Hitachi 561 recorder. The detector was operated at a potential of +0.85 V versus the Ag/AgCl reference electrode. A reversed phase column Nucleosil C-18, 200 x 4.6 ram ID (particle size 5 gm, pore size 100 Å) from HPLC Teknik HB (Robertsfors, Sweden) was used with a mobile phase of 0.1 M citric acid monohydrate (pH 2.45), 0.25 raM disodium EDTA, and 3.0% acetonitrile pumping at the flow rate 1.0 mL/min. The stock solutions of standards were prepared in ultrapure water at a concentration of 1 mM and stored at -20°C. Working standards (100-400 nM) were prepared on the day of assay by dilution with ultrapure water. Prior to injection the working standard solution was diluted 1:1 with 0.2 M perchloric acid. Aliquots of 500 µL CSF were transferred to conical plastic microvials. Afterwards 200 µL of internal standard (3-(4-hydroxyphenyl)-propionic acid 24 µM) and 100 µL of 0.8 M perchloric acid were added to the CSF. After careful mixing, the solution was filtered through a Millipore HV filter with pore size 0.45 µm and 100 µL and injected into the chromatographic system. The CSF monoamine metabolites were quantitated by comparison of standard and sample peak heights. The internal standard was used for correction of losses during the sample preparation.
3.13 STATISTICAL ANALYSES

3.13.1 Electrophysiology

The statistical software package GraphPad Prism 4.03 (GraphPad Software Inc., San Diego, CA, USA) was used. No differences were observed between the control groups and the groups treated with saline for 6 days in any of the tested parameters. For ease of presentation, all control data presented were pooled into one control group. Group comparisons of firing and percent burst firing activity of VTA dopamine neurons were carried out using the Kruskal–Wallis analysis of variance followed by Mann–Whitney U test. Statistical significance was considered for $p \leq 0.05$.

3.13.2 Microdialysis

No difference was observed between the control groups and the groups treated with saline for 6 days. For ease of presentation, all control data presented were pooled into one control group. The statistical software package GraphPad Prism 4.03 (GraphPad Software Inc., San Diego, CA, USA) was used. A p-value of $<0.05$ was considered statistically significant. Dopamine release is presented as the percent of baseline and the effect of amphetamine administration on dopamine release is analyzed using one-way analysis of variance (ANOVA), compared to predrug value, or two-way ANOVA for repeated measures (time x treatment) followed by Bonferroni post-hoc tests. Statistical significance was considered for $p \leq 0.05$.

3.13.3 Locomotor activity

GraphPad Prism 4 (GraphPad Software, Inc., San Diego, CA, USA) was used for the statistical analyses. In this study two subsets of mice were included; acute L-kynurenine treated mice with saline controls, and subchronic L-kynurenine treated mice with saline controls. No comparisons were performed between the two sets of mice. Locomotor activity data is presented in 5 min blocks. Percent peripheral activity is calculated as the ratio between the peripheral activity counts and the total horizontal activity counts for each animal. Unpaired t-test or two-way ANOVA (pretreatment x challenge) with following Bonferroni post hoc tests were used for the locomotor parameters horizontal activity, forward locomotion, rearing activity and percent peripheral activity. The locomotion parameter corner time as well as whole brain kynurenic acid content did not have normal distributions and were analyzed with the non-parametric Mann-Whitney U test. All tests on locomotor parameters were carried out on area under the curve (AUC) calculated for each mouse, with correction for baseline activity when applicable. Statistical significance was considered for $p \leq 0.05$.
3.13.4 Human cerebrospinal fluid studies

Group differences in the levels of kynurenic acid and cytokines were established using the non-parametric Mann–Whitney $U$ test (paper III, IV and V). In paper III, we tested for a normal distribution of residuals with the Anderson–Darling test for each of the groups and none of the groups deviated from normality ($p>0.25$), and linear regression analysis using the least squares method was used to study the relation between kynurenic acid level and age. In paper IV and V, we obtained correlations between variables using the Spearman rank correlation. In paper IV, CSF kynurenic acid values were transformed into natural logarithms in order to obtain normal distribution of residuals before linear regression analyses were performed. Logistic regression was used to analyze the influence of CSF kynurenic acid and age on the occurrence of psychotic symptoms (Y/N) and recent manic episodes (Y/N). All statistical analyses were performed using GraphPad Prism 4.0c (GraphPad Software, Inc.) and Statistical Package for the Social Sciences (SPSS) for Mac 19.0 (SPSS Inc., IBM, Somers, New York, USA). Statistical significance was assumed for $p \leq 0.05$. 
4 RESULTS AND DISCUSSION

4.1 THE AMPHETAMINE-INDUCED EFFECTS ON DOPAMINE TRANSMISSION AND BEHAVIOR AFTER ACUTE AND SUBCHRONIC ELEVATION OF BRAIN KYNURENIC ACID IN RODENTS

As previously mentioned (see section 1.4.1-2), dopaminergic hyperactivity has been suggested to cause both positive symptoms of schizophrenia and symptoms of mania and psychosis in bipolar disorder. Strong support for the dopamine hypothesis of schizophrenia comes from brain imaging studies showing an excessive amphetamine-induced dopamine release that correlates with the severity of positive symptoms in patients (Abi-Dargham et al., 1998; Breier et al., 1997; Laruelle et al., 1996). Although patients with bipolar disorder have been less studied in this regard, an increased behavioral response to amphetamine (transient hypomania) has been observed (Anand et al., 2000). Kynurenic acid, and its precursor kynurenine, is elevated in both the CSF and the postmortem brain of patients with schizophrenia (Erhardt et al., 2001a; Linderholm et al., 2010; Nilsson et al., 2005; Sathyasaikumar et al., 2010; Schwarcz et al., 2001), and increased cortical levels of kynurenine has also been reported in patients with bipolar disorder (Miller et al., 2006; 2008). Furthermore, increased CSF levels of kynurenic acid were recently found in suicide attempters with major depressive disorder (Linderholm, 2010). Kynurenic acid is an endogenous NMDA receptor antagonist and is known to cause increased midbrain dopaminergic activity (Erhardt and Engberg, 2002; Erhardt et al., 2001b; Linderholm et al., 2007; Nilsson et al., 2006).

In order to investigate the putative role of brain kynurenic acid in the pathophysiology of schizophrenia and bipolar disorder, we designed two studies to evaluate the amphetamine-induced effects on dopamine neurotransmission and behavior after elevation of brain kynurenic acid.

In the first paper, the amphetamine-induced effects on dopamine transmission following pharmacologically increased levels of brain kynurenic acid were investigated in male Sprague Dawley rats by in vivo extracellular single cell recordings of dopamine neurons in the VTA of anesthetized animals, and by measuring the dopamine output in nucleus accumbens with microdialysis in awake, moving animals. In order to increase brain kynurenic acid levels, rats were given a single dose (5 mg/kg, s.c.) or subchronic administration (90 mg/kg/day for six days, s.c. minipump) of L-kynurenine. Acute and subchronic L-kynurenine treatment resulted in a 3- and 2-fold increase, respectively, in whole brain kynurenic acid levels compared to saline controls.

In the second paper, spontaneous and amphetamine-induced locomotor activity was measured in male C57BL/6 mice with increased brain kynurenic acid levels. Mice were pretreated with an acute dose (10 mg/kg, i.p.) or subchronic administration (100 mg/kg, i.p. twice daily for six days) of L-kynurenine, resulting in a 2- and 4-fold increase, respectively, in whole brain kynurenic acid levels compared to saline controls.
4.1.1 Effects on amphetamine-induced dopamine release (paper I)

The accumbal extracellular dopamine levels were measured by microdialysis and HPLC with electrochemical detection and presented as percent of baseline levels. Following 2 mg/kg amphetamine administration i.p., extracellular dopamine levels increased to a maximum of 374% in control rats, peaking 60 minutes after injection. A slightly higher increase (461%) was observed in rats with acute elevation of brain kynurenic acid, but this effect was non-significant compared to controls. Animals with subchronic elevation of brain kynurenic acid, however, had a clearly potentiated dopamine release, with a maximum increase of 814% of baseline, 60 minutes after amphetamine injection (figure 3). These results suggest that subchronic, but not acute, elevation of brain kynurenic acid in the rat produce changes in the dopamine system leading to an amphetamine-induced hyperresponsiveness similar to that observed in patients with schizophrenia. Further, acute elevation of kynurenic acid, by systemic administration of 5 mg/kg L-kynurenine s.c., was associated with a trend towards reduced (25%) striatal dopamine output. This finding is in line with previous studies showing decreased extracellular dopamine following acute increase of brain kynurenic acid in rat striatum (Capone et al., 2008; Rassoulpour et al., 2005).

Figure 3. Effects of amphetamine (2 mg/kg i.p.) administration on dopamine (DA) output in the nucleus accumbens in awake, freely moving rats. Each point represents the mean±SEM percent of baseline. *p<0.05, **p<0.01, ***p<0.001 compared to predrug value (one-way ANOVA followed by Bonferroni multiple comparison test. +p<0.05, ++p<0.01 between-group comparisons [two-way ANOVA for repeated measurements (time x treatment) followed by Bonferroni post-hoc tests].
4.1.2 Investigation of amphetamine-induced dopamine release in rat using small animal PET

In order to follow-up the microdialysis study, we conducted *in vivo* brain imaging experiments in anesthetized male Sprague Dawley rats, untreated or treated with subchronic L-kynurenine (90 mg/kg/day for six days, s.c. minipump), using small animal PET. Amphetamine (2 mg/kg, i.p.) induced a decreased striatal binding of the dopamine D2-receptor specific $[^{11}C]RAC$, an effect, in all probability, secondary to increased competition for the binding site due to endogenous dopamine release (Hume et al., 1992; Seeman et al., 1989). Consistent with the microdialysis results in paper I, initial pilot results (n=3) indicated a larger striatal displacement of $[^{11}C]RAC$ by amphetamine after subchronic elevation of brain kynurenic acid compared to saline controls (data not shown). Further analyzes of $[^{11}C]RAC$ tracer binding in the rat brain following subchronic elevation of kynurenic acid are ongoing.

4.1.3 Effects on dopaminergic firing activity (paper I)

The effects of elevated brain kynurenic acid on spontaneous firing rate and spike distribution are demonstrated in table 1. Previous studies show that acute (200 mg/kg; Linderholm et al., 2007), and subchronic (20 mg/kg/day together with 10 mg/kg/day probenecid for 14 days; Nilsson et al., 2006) systemic L-kynurenine administration increase dopamine firing rate and burst firing activity in the VTA. In the present study, a single dose of 5 mg/kg L-kynurenine increased burst firing activity but did not change the firing rate of VTA dopamine neurons, a discrepancy likely related to the low dose of L-kynurenine used in this study. Subchronic (90 mg/kg/day, s.c., for six days) pretreatment increased both firing rate and burst firing activity, in agreement with previous observations.

**Table 1.** Effects of L-kynurenine pretreatment on the firing rate and spike distribution of dopamine neurons in the VTA.

<table>
<thead>
<tr>
<th>Controls</th>
<th>Acute L-kynurenine (5 mg/kg; s.c., 60 min)</th>
<th>Subchronic L-kynurenine (90 mg/kg/day; s.c., for six d)</th>
</tr>
</thead>
<tbody>
<tr>
<td>(33 neurons)</td>
<td>(32 neurons)</td>
<td>(19 neurons)</td>
</tr>
<tr>
<td><strong>Firing rate (Hz)</strong></td>
<td>4.3 ± 0.3</td>
<td>4.8 ± 0.4</td>
</tr>
<tr>
<td>Mean % burst firing</td>
<td>19.7 ± 39</td>
<td>37.2 ± 5.9*</td>
</tr>
</tbody>
</table>

Values represent mean±SEM from 24 control rats, 11 rats treated with acute L-kynurenine and 13 rats subchronically treated with L-kynurenine. Statistics: *p<0.05, **p<0.01 vs. corresponding control value (Kruskal-Wallis analysis of variance followed by Mann-Whitney U test).

Amphetamine increases extracellular dopamine concentrations by causing an efflux of vesicular and cytoplasmic dopamine through the DAT (see section 1.5.4). The synaptic increase of dopamine triggers feedback loops aimed at suppressing dopaminergic firing activity. The negative feedback is mediated by both somatodendritic dopamine D2 autoreceptors and postsynaptic dopamine D1 receptors located on GABAergic afferents.
impinging upon the soma of dopamine neurons (see White, 1996). In order to investigate the effects of brain kynurenic acid on the dopamine system, the firing pattern of dopamine VTA neurons under the influence of amphetamine was studied.

Systemic administration of amphetamine was found to dose-dependently reduce firing rate and percent burst firing activity of VTA dopamine neurons. In control rats, the firing rate of 9 out of 13 VTA dopamine neurons was totally suppressed following administration of 1.6 mg/kg amphetamine (figures 4 and 5a). The dopamine neurons that continued to fire following administration of 1.6 mg/kg amphetamine were quiescent after administration of 3.2 mg/kg (two neurons) or 6.4 mg/kg (two neurons). Acute elevation of brain kynurenic acid did not change the inhibitory action of amphetamine (figure 4). However, in rats with subchronic increase in brain kynurenic acid levels, larger doses of amphetamine were required to induce inhibition of firing rate and percent burst firing activity (figures 4 and 5b). Thus, 1.6 mg/kg amphetamine was not able to totally suppress firing in any dopamine neurons recorded from in these rats. Following 12.8 mg/kg amphetamine two neurons were still firing and bursting, one of them even at the dose 25.6 mg/kg and this neuron was not quiescent until apomorphine (100 mg/kg i.v.) was administered. These results emphasize that adaptive changes take place in a situation of prolonged exposure to high levels of brain kynurenic acid, causing attenuated negative feedback control in the dopamine system. One contributing factor to the excessive dopamine efflux seen in these animals might thus be the reduced responsiveness of VTA dopamine neurons to the inhibitory actions of amphetamine.

Figure 4. (Above) Cumulative dose–response curves illustrating (a) the firing rate, and (b) the burst firing activity of ventral tegmental area (VTA) dopamine neurons following i.v. injections of d-amphetamine in control rats and L-kynurenine pretreated rats. Each point represents the mean±SEM obtained from 8–15 neurons. *p<0.05, between-group comparisons (Kruskal–Wallis analysis of variance followed by Mann–Whitney U test).

Figure 5. (Left) In vivo extracellular single cell recordings from dopamine neurons in the ventral tegmental area (VTA) following intravenous administration of d-amphetamine in cumulative doses (0.2+0.2+0.4+0.8+1.6+3.2 mg/kg at arrows) in (a) a control rat, and (b) a rat after subchronic treatment with L-kynurenine (90 mg/kg/day, s.c., for six days).
4.1.4 Effects on locomotor activity (paper II)

The open field test was used to assess effects of increased brain kynurenic acid on spontaneous and amphetamine-induced open field locomotor activity. When placed inside the open field test box, the animal’s desire to explore the environment will weigh against the innate fear of being exposed in an open field. The acute increase of brain kynurenic acid caused increased percent peripheral activity and time spent in the corners compared to control animals (figure 6a,b). This type of behavior (i.e., avoidance of the centre of the open field arena) is usually interpreted as anxiety related.

Further, acute elevation of brain kynurenic acid resulted in lower horizontal activity, forward locomotion and rearing activity compared to saline treated control mice (figure 6c-e). In vivo microdialysis on rats has previously demonstrated reduced dopamine output in the striatum following acute elevation of brain kynurenic acid (Rassoulpour et al., 2005). A decrease in extracellular dopamine levels might explain the tendencies towards lower locomotor activity observed in paper II. In mice with subchronic elevation of brain kynurenic acid, no changes were observed in the spontaneous locomotor activity compared to saline treated controls (data not shown).

![Graphs showing effects on locomotor activity](image)

**Figure 6.** Effects on corner time (A), % peripheral activity (B) horizontal activity (C), forward locomotion (D) and rearing activity (E) for mice that received acute administration of either L-kynurenine (Kyn) or saline (Sal) at time point 0. Graphs show open field activity in 5 min intervals during 60 min following injection. *p<0.05 vs. saline controls.
The administration of d-amphetamine (5 mg/kg, i.p.) induced an increased amount of horizontal activity, forward locomotion and rearing activity in both L-kynurenine pretreated and control animals, with a peak 20-30 minutes post injection. Mice with acute elevation of brain kynurenic acid showed an amphetamine-induced locomotor response of the same magnitude as control animals (data not shown). Subchronic elevation, however, produced increased horizontal activity, forward locomotion, and rearing activity in response to amphetamine challenge, compared to saline controls (figure 7).

**Figure 7.** Effects of d-amphetamine (Amph; 5 mg/kg, i.p.) or saline (Sal) challenge (indicated by arrow) on horizontal activity (A), forward locomotion (B) and rearing activity (C) in mice that received subchronic pretreatment of either L-kynurenine (Kyn) or Sal for six days. Graphs on the left show open field activity in 5 min intervals. Graphs on the right show total area under the curve (AUC) of the 0–90 min trial period for the same groups of mice as shown to the left. Two-way ANOVA: (A) Pretreatment (Sal/Kyn): F=4.25, p=0.05; Challenge (Sal/Amph): F=108, p<0.001; Interaction: F=8.35, p=0.01 (B) Pretreatment (Sal/Kyn): F=4.98, p=0.04; Challenge (Sal/Amph): F=110, p<0.001; Interaction: F=10.50, p=0.005 (C) Pretreatment (Sal/Kyn): F=4.47, p=0.05; Challenge (Sal/Amph): F=132, p<0.001; Interaction: F=8.38, p=0.01, followed by Bonferroni post hoc tests; **p<0.01, +++p<0.001 compared to respective saline challenge.
Results and Discussion

The observation that subchronic, but not acute, elevation of brain kynurenic acid caused increased amphetamine-induced locomotor activity is in agreement with the microdialysis results in paper I (see section 4.1.1). These findings are also consistent with studies from other laboratories, showing increased amphetamine-induced locomotor response in animals with prolonged pretreatment with the NMDA receptor antagonist PCP (Beninger et al., 2010; Jentsch et al., 1998b).

In summary, these results demonstrate that subchronic elevation of brain kynurenic acid in rodents produces increased VTA dopamine firing activity and altered dopaminergic response to amphetamine. Thus, both larger accumbal dopamine release and augmented locomotor response was observed following amphetamine administration. The excessive dopamine release might, at least in part, be related to a reduced feedback inhibition of VTA dopamine firing activity following amphetamine administration.

4.2 CEREBROSPINAL FLUID KYNURENIC ACID IN PATIENTS WITH BIPOLAR DISORDER

As previously mentioned, bipolar disorder is characterized by recurrent episodes of manic or depressive mood, interspersed with periods of euthymia (see section 1.2.2). Manic episodes can also feature psychotic symptoms such as hallucinations and delusions and may be indistinguishable from acute psychosis in schizophrenia. Further, cognitive impairments are core features of both bipolar disorder and schizophrenia. Similarities in clinical characteristics as well as treatment and genetic liability (see section 1.3) are indicative of a partially shared pathophysiology of these disorders.

Considering that kynurenic acid has the potential to affect dopamine transmission and increased brain levels have been established in patients with schizophrenia (cf. Introduction), we analyzed the kynurenic acid content in CSF collected from euthymic patients diagnosed with bipolar disorder and compared these levels with their clinical manifestations. In the first study (paper III), CSF kynurenic acid levels of male euthymic patients diagnosed with bipolar disorder type I (n=18) or II (n=13) were compared with levels of healthy male volunteers (n=23). In the second study (paper IV), CSF kynurenic acid levels of male (n=21) and female (n=34) patients diagnosed with bipolar disorder type I were analyzed with regard to their history of mood episodes and symptoms. For ethical reasons, patients continued with their medication during the CSF sampling.

4.2.1 Kynurenic acid in patients and healthy volunteers (paper III)

Euthymic patients with bipolar disorder had 51% higher CSF kynurenic acid levels (1.71 nM ±0.13 SEM) than healthy volunteers (1.13 nM ±0.09 SEM; figure 8a). There was no difference between CSF kynurenic acid levels of patients with the type I (1.77 nM ±0.17 SEM) and the type II (1.63 nM ±0.21 SEM) diagnostic subtypes. These results greatly resemble the CSF levels found in patients with schizophrenia, with mean levels ranging between 1.45 and 2.03 nM with an average increase of 53%
compared to controls (Erhardt et al., 2001a; Linderholm et al., 2010; Nilsson et al., 2005). Further, CSF kynurenic acid levels were found to increase with age in the bipolar disorder group but not in healthy volunteers (figure 8b). In coherence, previous studies have reported a positive correlation between CSF levels of kynurenic acid and age in patients with schizophrenia (Nilsson et al., 2005) and individuals with acute severe headache (Kepplinger et al., 2005), but not in healthy volunteers (Nilsson et al, 2005; Heyes et al., 1992).

Figure 8a. Kynurenic acid (KYNA) content in the cerebrospinal fluid (CSF) of healthy volunteers and individuals with bipolar disorder. Each point represents the concentration of kynurenic acid in a single CSF sample. The line indicates the mean of each group. Mann–Whitney U test; $U=181$, **$p<0.01$.

Figure 8b. Linear regression of the amount of cerebrospinal fluid (CSF) kynurenic acid (KYNA) and age of healthy volunteers (open circles) and individuals with bipolar disorder (closed circles). A significant correlation between kynurenic acid and age was observed in male patients with bipolar disorder ($y=0.0397x + 0.27$, $r=0.49$, **$p=0.005$).
4.2.2 Associations between kynurenic acid and symptoms of bipolar disorder (paper IV)

The CSF kynurenic acid levels, age, and history of mood episodes and psychotic symptoms are summarized in table 2. There was no correlation between CSF kynurenic acid levels and gender (data not shown). However, CSF kynurenic acid levels significantly correlated with age (Spearman’s $r=0.395$, $p=0.003$, $n=55$).

Table 2. Subject overview. All values are given as mean±SD.

<table>
<thead>
<tr>
<th></th>
<th>Male</th>
<th>Female</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>n</td>
<td>21</td>
<td>34</td>
<td>55</td>
</tr>
<tr>
<td>CSF kynurenic acid (nM)</td>
<td>1.8 ±0.9</td>
<td>1.9 ±1.1</td>
<td>1.9 ±1.0</td>
</tr>
<tr>
<td>Age (years)</td>
<td>41 ±14</td>
<td>37 ±14</td>
<td>39 ±14</td>
</tr>
<tr>
<td>Lifetime number of manias</td>
<td>3.0 ±3.0</td>
<td>3.4 ±3.5</td>
<td>3.3 ±3.3</td>
</tr>
<tr>
<td>Lifetime number of depressions</td>
<td>10.3 ±13.4</td>
<td>6.7 ±10.5</td>
<td>8.0 ±11.7</td>
</tr>
<tr>
<td>Psychotic features (Y/N)</td>
<td>(14/7)</td>
<td>(29/5)</td>
<td>(43/12)</td>
</tr>
</tbody>
</table>

Patients with a history of psychotic symptoms had higher levels of CSF kynurenic acid (2.0 nM ±0.2 SEM, $n=43$) compared to patients without a history of psychotic features (1.3 nM ±0.2 SEM, $n=12$; Mann-Whitney U test, $U=138$, $p=0.01$; figure 9). Logistic regression analysis with age as covariate confirmed a significant correlation between CSF kynurenic acid and the occurrence of psychotic features (Y/N) with an odds ratio of 4.9 ($p=0.03$, $n=55$).

The total lifetime number of manic and depressive episodes were significantly correlated with the CSF kynurenic acid levels (Spearman’s $r=0.285$, $p=0.04$). When analyzed separately, the lifetime number of manias and the lifetime numbers of depressions indicated trends towards a correlation with CSF kynurenic acid levels ($r=0.23$, $p=0.1$ and $r=0.25$, $p=0.06$, respectively). However, the lifetime number of episodes were not normally distributed and could not be transformed into a normal distribution due to the large skewness in the data. Further analyses of the relative contributions of kynurenic acid and age with respect to the number of lifetime episodes using multiple linear regression analyses were therefore not relevant. When the opposite scenario was analyzed, the total lifetime number of depressions ($\beta^C=-0.14$, $p=0.3$) or manias ($\beta^C=0.21$, $p=0.11$) did not explain the variance observed in CSF kynurenic acid levels (transformed to natural logarithms for normal distribution of residuals, and age as covariate). However, kynurenic acid levels were significantly correlated with the recent occurrence of manic episodes (logistic regression with age as covariate; odds ratio=4.4, $p=0.03$, $n=34$). No correlation was found between recent episodes of depression and kynurenic acid. Considering the neurophysiologic properties of kynurenic acid, increased CSF kynurenic acid might drive dopaminergic activity, which in turn may increase the risk of developing symptoms of mania and/or psychosis. This hypothesis is further supported by the observed correlation between CSF kynurenic acid and the dopamine metabolite HVA in these patients (figure 10).
Figure 9. Cerebrospinal fluid (CSF) levels of kynurenic acid (KYNA) in patients with bipolar disorder type I. Open circles represent patients without any lifetime occurrence of psychotic features, n=12, mean age 37±14. Closed circles represent patients with a lifetime occurrence of psychotic features, n=43, mean age 39±14. Mann-Whitney U test; U=138, **p=0.01.

4.2.3 Additional results

CSF kynurenic acid levels were significantly correlated with CSF levels of the dopamine metabolite HVA in the bipolar disorder type I patients in paper IV (Spearman’s r=0.616, p<0.0001, n=54; figure 10).

Figure 10. Cerebrospinal fluid (CSF) levels of kynurenic acid (KYNA) and homovanillic acid (HVA) in patients with bipolar disorder type I. Spearman’s r= 0.616, **p<0.0001, n=54.

In summary, paper III and IV show that the CSF kynurenic acid content is increased in euthymic bipolar disorder patients compared to healthy controls, in an age-dependent manner. Furthermore, CSF kynurenic acid levels correlate with the history of psychotic symptoms as well as the recent occurrence of a manic episode in patients with bipolar disorder I. Given the positive correlation between CSF kynurenic acid content and the dopamine metabolite HVA, a possible explanation is that increased kynurenic acid drives dopaminergic activity and thereby increases the risk of developing symptoms of mania and psychosis.
4.3 CEREBROSPINAL FLUID CYTOKINES IN PATIENTS WITH BIPOLAR DISORDER

In recent years a role for the immune system in the pathogenesis of psychiatric diseases has gained increased attention. Many investigators have focused on cytokines, proteins that directly initiate and control immunological responses, and several alterations have been reported in plasma/serum of patients with bipolar disorder (see section 1.4.6). However, to our knowledge, no direct analyses of CSF cytokines have been performed in patients with bipolar disorder. My collaborators recently demonstrated a selective activation of brain IL-1β in schizophrenia (Söderlund et al., 2009). In order to investigate the possible involvement of cytokines in the pathophysiology of bipolar disorder we analyzed the CSF content of cytokines (IL-1β, IL-2, IL-4, IL-5, IL-6, IL-8, IL-10, granulocyte-macrophage colony-stimulating factor [GM]-CSF, IFN-γ and TNF) in male individuals diagnosed with bipolar type I (n=15) or II (n=15) and correlated the levels of these cytokines to their history of mood episodes (paper V).

4.3.1 Cytokine profile and correlation with previous mood episodes (paper V)

Three of the cytokines analyzed, IL-1β, IL-6 and IL-8, were consistently found in detectable levels in all patients and healthy volunteers. The results showed increased concentrations of IL-1β in lumbar CSF of euthymic patients with bipolar disorder (4.2 pg/mL ±0.5 SEM) compared to healthy volunteers (0.8 pg/mL ±0.04 SEM; Mann-Whitney U test, U=118, p<0.001; see figure 11). This finding is consistent with a recent postmortem study showing elevated mRNA levels of IL-1β in the cortex of patients with bipolar disorder (Rao et al., 2010). Further, as demonstrated in figure 11, patients with recent manic or hypomanic episodes (≥1 during the year preceding CSF sampling) had higher levels of IL-1β (6.2 pg/mL ±0.8 SEM, n=9) than those without a history of recent manic or hypompanic episodes (3.1 pg/mL ±1.0 SEM, n=10; Mann-Whitney U test, U=20, p=0.04). The patients with or without recent manic/hypompanic episodes did not differ in diagnostic subtype, age or the lifetime number of manic/hypompanic episodes. In contrast, recent episodes of depression (≥1 in the year preceding CSF sampling) did not correlate to CSF cytokine levels (data not shown). Thus, while a CSF increase of the pro-inflammatory cytokine IL-1β was apparent in all euthymic subjects compared to healthy controls, the highest levels of this pro-inflammatory cytokine was found in patients with a recent state of mania/hypomania, which might indicate fluctuations in cytokine levels over time or in relation to different mood states in bipolar disorder. In accordance with this notion, the total lifetime number of depressive episodes were found to be negatively correlated with CSF levels of IL-1β (Spearman correlation=−0.52, p=0.048, n=15). Moreover, CSF levels of IL-6 were found to be decreased in patients (1.5 pg/mL ±0.2 SEM) compared to healthy volunteers (2.6 pg/mL ±0.2 SEM; Mann-Whitney U test, U=171.5, p<0.001; see figure 12). IL-8 levels (75 pg/mL ±10 SEM) did not differ from those in healthy volunteers (90 pg/mL ±3 SEM). No associations were found between the cytokine levels measured and diagnostic subtype, ongoing medication or smoking (data not shown) in patients with bipolar disorder. Furthermore, the cytokine levels did not correlate with age or CSF kynurenic acid levels in patients or in healthy volunteers (data not shown).
Figure 11. Interleukin (IL)-1β in cerebrospinal fluid (CSF) of healthy volunteers and patients with bipolar disorder, with or without recent manic/hypomanic episodes (during the year preceding CSF sampling). Each point represents the concentration of IL-1β in a single CSF sample. Horizontal lines show mean values for each group. Mann–Whitney U test, *p<0.05, ***p<0.001.

Figure 12. Interleukin (IL)-6 in cerebrospinal fluid (CSF) of healthy volunteers and patients with bipolar disorder. Each point represents the concentration of IL-6 in a single CSF sample. Horizontal lines show mean values for each group. Mann–Whitney U test, ***p<0.001.

In summary, euthymic patients with bipolar disorder have an aberrant CSF cytokine profile, with increased levels of IL-1β and decreased levels of IL-6, compared to healthy volunteers. Recent episodes of mania and/or hypomania are associated with high levels of IL-1β, while the lifetime number of depressions are negatively correlated with IL-1β. Although the causality of these findings needs further investigation, cytokine activation may play a role in the pathophysiology of bipolar disorder.
5 GENERAL DISCUSSION

Since the discovery of kynurenic acid in the human brain (Moroni et al., 1988; Turski and Schwarcz, 1988) and the initial findings of increased levels in the CSF and cortex of patients with schizophrenia (Erhardt et al., 2001a; Schwarcz et al., 2001), the role of kynurenic acid in brain physiology has been extensively studied. Kynurenic acid antagonizes the glycine site of the NMDA receptor and the α7nACh receptor with IC50 values in the low micromolar range (~7-15 µM; cf. Introduction). The relatively low kynurenic acid content found in brain tissue (about 6 nM in mice, 20 nM in rat and 1µM in human; Moroni et al., 1988; Turski and Schwarcz, 1988) therefore initially raised questions regarding the physiological significance of the compound. However, kynurenic acid is synthesized in and released by astrocytes, cells that are intimately arranged with pre- and postsynaptic elements and actively take part in synaptic transmission by releasing a variety of gliotransmitters and neuromodulators (cf. Introduction). Local concentrations of kynurenic acid within the synaptic clefts therefore most likely exceed the levels detected in brain tissue. Indeed, several studies have demonstrated that endogenous kynurenic acid is able to interact with receptors in the brain in vivo as both increased and decreased levels cause profound alterations in firing activity, neurotransmitter release and behavior in animals. Specifically, acute elevation of brain kynurenic acid, by systemic administration of L-kynurenine or by regional infusion of nanomolar concentrations of kynurenic acid, is associated with increased firing activity of midbrain dopamine neurons, reduced striatal, hippocampal and cortical levels of glutamate, reduced striatal dopamine levels, tendencies towards lower cortical acetylcholine levels, cognitive deficits, and impaired prepulse inhibition (Erhardt et al., 2004; Konradsson-Geuken et al., 2010; Linderholm et al., 2007; Pocivavsek et al., 2011; Rassoulpour et al., 2005; Wu et al., 2010; Chess et al., 2007; Zmarowski et al., 2009). Reversely, reduced endogenous kynurenic acid is associated with dampened firing activity of midbrain dopamine neurons, increased hippocampal and cortical levels of glutamate, increased cortical acetylcholine levels, and improved cognitive performance (Konradsson-Geuken et al., 2010; Pocivavsek et al., 2011; Potter et al., 2010; Schwieler et al., 2006; 2008; Wu et al., 2010; Zmarowski et al., 2009). In the past few years it has become evident that brain kynurenic acid also serve as a biological marker of brain immune activation. Indeed, CSF kynurenic acid is markedly elevated following CNS infections, such as those caused by human immunodeficiency virus or tick-borne encephalitis (Atlas et al., 2007; Schwieler et al., 2007). Additionally, IDO, the rate-limiting enzyme of the kynurenine pathway, is important in the control of immune responses (King and Thomas, 2007).

In the experimental part of this work, the physiological effects of increased brain kynurenic acid were investigated with focus on the typical hyperdopaminergic alterations associated with symptoms of mania and psychosis. Amphetamine, a drug causing massive dopamine release by reversing the function of terminal DAT, induces symptoms of euphoria, alertness and over-confidence, which are all typical symptoms of mania, in healthy volunteers. High doses and/or prolonged use are also associated with symptoms of psychosis. Patients with schizophrenia respond to amphetamine with
an excessive striatal dopamine release, which is correlated to the exacerbation of positive symptoms (Abi-Dargham et al., 1998; Laruelle et al., 1996). Further, patients with bipolar disorder show an increased hypomanic response to amphetamine compared to healthy controls (Anand et al., 2000). Consistent with these clinical observations, present results showed that subchronic elevation of brain kynurenic acid caused an excessive amphetamine-induced accumbal dopamine release and increased the amphetamine-induced behavioral response, in terms of locomotor activity, in rodents. The dopamine neurons of these animals also showed signs of reduced feedback inhibition, as higher doses of amphetamine were required to silence the VTA dopamine firing activity. In contrast, acute elevation of brain kynurenic acid did not affect the amphetamine-induced dopamine release, behavior, or the amphetamine-induced inhibition of VTA dopamine firing activity, indicating that these changes in dopamine transmission require adaptive changes.

<table>
<thead>
<tr>
<th></th>
<th>Acute elevation of brain kynurenic acid</th>
<th>Subchronic elevation of brain kynurenic acid</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>Spontaneous midbrain dopamine firing activity</td>
<td>Increased</td>
<td>Increased</td>
<td>(Erhardt et al., 2001b; Erhardt and Engberg 2002; Nilsson et al., 2006; Schwieler et al., 2006, 2008; Linderholm et al., 2007; present results)</td>
</tr>
<tr>
<td>Basal extracellular dopamine levels (striatal)</td>
<td>Decreased</td>
<td>Increased</td>
<td>(Capone et al., 2008; Rassoulpour et al., 2005)</td>
</tr>
<tr>
<td>Spontaneous locomotor activity</td>
<td>Decreased (trend)</td>
<td>Unaltered</td>
<td>(Present results)</td>
</tr>
<tr>
<td>d-amphetamine effect on midbrain dopamine firing activity</td>
<td>Unaltered</td>
<td>Reduced feedback inhibition</td>
<td>(Present results)</td>
</tr>
<tr>
<td>d-amphetamine effect on extracellular dopamine levels (accumbal)</td>
<td>Unaltered</td>
<td>Excessive release</td>
<td>(Present results)</td>
</tr>
<tr>
<td>d-amphetamine effect on locomotor activity</td>
<td>Unaltered</td>
<td>Exaggerated response</td>
<td>(Present results)</td>
</tr>
</tbody>
</table>

The effects of brain kynurenic acid on dopamine neurotransmission and behavior are complex and dependent on duration (see table 3). During acute elevation, kynurenic acid exerts dual and opposite effects on mesolimbic dopamine neurotransmission by increasing firing activity of midbrain dopamine neurons, while simultaneously decreasing striatal dopamine release (see table 3). The effect on dopamine firing activity has previously been demonstrated to be mediated through the antagonistic
properties by kynurenic acid on NMDA receptors, preferentially at the glycine co-
agonistic site (Erhardt and Engberg, 2002; Linderholm et al., 2007). The firing activity
of dopamine neurons in the VTA is regulated by intrinsic and extrinsic (projecting from
e.g., nucleus accumbens and VTA) GABAergic afferents that negatively influence
dopamine excitability (Beart and McDonald, 1980; Kalivas, 1993; Walaas and
Fonnum, 1980). GABAergic interneurons are particularly sensitive to NMDA receptor
blockade (Grunze et al., 1996) and systemic administration of non-competitive NM
DA receptor antagonists such as PCP and MK-801 have been shown to inhibit the ac-
tivity of VTA GABAergic interneurons (Zhang et al., 1993). Thus, the effects on dopamine
firing by kynurenic acid are likely caused by a reduced GABAergic tone in the VTA,
leading to disinhibition of dopamine neurons. In contrast, the effect on striatal
dopamine release induced by acute elevation of kynurenic acid is mediated via
antagonism of α7nACh receptors, located presynaptically on glutamatergic afferents in
the striatum (Rassoulpour et al., 2005). Thus, blockade of α7nACh receptors causes
reduced striatal levels of extracellular glutamate and, in turn, reduced AMPA receptor-
mediated augmentation of dopamine release (Wu and Schwarz, 2009). Present results
show that acute elevation of brain kynurenic acid was associated with a trend towards
reduced (25%) striatal dopamine output, in line with previous findings (Rassoulpour et
al., 2005). Further, mice with acute elevation of endogenous brain kynurenic acid levels
showed anxiety-related behavior and tendencies of reduced spontaneous locomotor
activity in the open field paradigm presently used. A decrease in striatal dopamine
output, as seen in rats, may explain the tendency towards reduced locomotor activity
observed in the present study.

Subchronic elevation of brain kynurenic acid resulted in increased firing rate and
burst firing of VTA dopamine neurons, in agreement with previous data (Nilsson et al.,
2006). In contrast to the acute effects of elevated brain kynurenic acid, subchronic
elevation is associated with an increase in striatal dopamine output (Capone et al.,
2008). In the present study, spontaneous locomotor activity was unaltered in mice
following subchronic elevation of brain kynurenic acid. Unaltered behavioral response
in a situation where basal levels of striatal dopamine are increased might suggest that
desensitization, in terms of decreased dopamine receptor expression, or signaling, has
occurred. Further, subchronic elevation of brain kynurenic acid resulted in excessive
amphetamine-induced dopamine release in nucleus accumbens and caused increased
amphetamine-induced locomotor response in rodents. The mechanisms underlying the
presently observed amphetamine hyperresponsiveness are unclear. However, one might
speculate that a prolonged increase in dopamine firing without a simultaneous increase
in striatal dopamine release would lead to a build-up of dopamine content over time.
When challenged with amphetamine, vesicular and cytoplasmic dopamine exits the
neuron through reversed transport via DAT. An exaggerated amphetamine-induced
dopamine release might thus mirror an excessive dopamine content in these neurons.
Moreover, subchronic elevation of kynurenic acid was found to attenuate feedback
inhibition of VTA dopamine activity. Released dopamine triggers D1 receptor-mediated
negative feedback loops and somatodendritic inhibitory D2-like autoreceptors, causing
a dose-dependent decrease of dopaminergic firing in response to amphetamine (Clark
and Chiodo, 1988). VTA dopamine neurons recorded from animals with subchronic
elevation of brain kynurenic acid showed less sensitivity in this regard, and higher
doses of amphetamine were required to suppress their firing. This finding further
indicates adaptive changes as a result of prolonged elevation of brain kynurenic acid. A hampered dopamine feedback signaling in these animals might also explain the increase in basal striatal dopamine levels previously reported (Capone et al., 2008). Adaptive changes in response to prolonged kynurenic acid exposure may also involve non-dopamine receptors. As previously mentioned, the decrease in striatal extracellular dopamine observed following acute elevation of kynurenic acid is mediated through inhibition of \( \alpha \text{7nACh} \) receptors in the striatum. This receptor is known to rapidly adapt following agonist exposure (Anand et al., 1993; Marks et al., 1985) and changes in receptor expression or sensitivity should also be considered following subchronic exposure to the antagonist kynurenic acid. Moreover, kynurenic acid was recently recognized as the endogenous ligand of the G protein-coupled receptor 35 (Wang et al., 2006). This receptor is known to be expressed in cultured human and rat astrocytes (unpublished data from our laboratory) and rat dorsal root ganglion neurons (Ohshiro et al., 2008). Although its functional role remains to be established, activation of this receptor should not be disregarded when considering the adaptive changes following long-term elevation of brain kynurenic acid.

In the present studies, systemic administration of L-kynurenine was used in order to increase endogenous cerebral production of kynurenic acid. L-kynurenine is a key compound of the kynurenine pathway and can also be catabolized to the neuroactive compounds 3-HK and quinolinic acid. Although kynurenic acid was the only kynurenine metabolite measured in the present studies, increased levels of 3-HK and quinolinic acid may have contributed to the observed effects of L-kynurenine treatment. However, 3-HK is a free-radical generator (Eastman and Guilarte, 1989) and appears not to directly interact with glutamatergic or dopaminergic processes, making it less likely to participate in the effects induced by L-kynurenine in the present studies. Quinolinic acid is an NMDA receptor agonist and increased brain formation of this compound might lead to lesions and neuronal cell death (Stone, 1993). The two branches of the kynurenine pathway leading to formation of the neuroprotective kynurenic acid or the excitotoxic quinolinic acid seem to be functionally segregated in the brain and the enzymes involved in either branch differ both in their \( K_m \) values and cellular expression. Thus, KAT II (the predominant catalyst of brain kynurenine acid synthesis) has a \( K_m \) in the low millimolar range (Han et al., 2008; Okuno et al., 1991b; Passera et al., 2011), while \( K_m \) values for KMO, KYNU and 3-hydroxyanthranilate 3,4-dioxygenase (HAAO) enzymes (leading to the production of 3-HK and quinolinic acid) are in the low micromolar range and thus more easily saturated (Bender and McCleanor, 1982; Lima et al., 2009; Saito et al., 1993; Zhang et al., 2005). Further, KAT II is almost exclusively expressed by astrocytes (the most abundant glial cell type), which appear to lack the expression of KMO (Guidetti et al., 2007b; Guillemin et al., 2001; Kiss et al., 2003). In contrast, microglia are the main source of quinolinic acid production in the brain and have limited expression of KAT enzymes (Lehrmann et al., 2001). A recent study has also shown that the constitutive expression of KAT II is 8-folds higher than KMO in cortex and 20-fold higher in hippocampus in rats (Connor et al., 2008). In line with this, intrastratial infusion of \(^{3}\text{H}\text{-kynurenine in rats in vivo}\) is associated with higher de novo synthesis of kynurenic acid than quinolinic acid, with a ratio of 1.0 to 0.2 (Amori et al., 2009; Guidetti et al., 1995), and the quinolinic acid branch of the pathway is easily saturated following infusion of increasing concentrations of \(^{3}\text{H}\text{-kynurenine}\) (Guidetti et al., 1995). Thus, kynurenic acid should be
General Discussion

the main product following L-kynurenine treatment in the present work. In support of this, administration of L-kynurenine has been found to attenuate quinolinic acid-induced excitotoxicity in the rat, a finding in all probability related to a predominating production of kynurenic acid (Nozaki and Beal, 1992; Santamaria et al., 1996; Vecsei et al., 1992). Similarly, in mice, the excitotoxic effect of quinolinic acid is only enhanced when endogenous levels of kynurenic acid had been lowered (Sapko et al., 2006). In addition, in line with the results from L-kynurenine treated rodents in this thesis, increased dopamine firing activity has previously been observed in rats treated with either L-kynurenine or the KMO inhibitor PNU 156561A, which increase kynurenic acid by blocking the production of 3-HK (Erhardt et al., 2001b; Linderholm et al., 2007). Moreover, also the NMDA receptor antagonists PCP and MK-801 (French, 1994; French et al., 1993) and the specific glycine site NMDA receptor antagonist 7-chloro-kynurenic acid (Linderholm et al., 2007) increase midbrain dopamine firing. Most likely, increased brain levels of kynurenic acid should therefore account for the effects on dopamine neurotransmission and behavior induced by L-kynurenine pretreatment in the present thesis.

In the clinical part of this thesis, the CSF contents of kynurenic acid and various cytokines were analyzed in euthymic patients with bipolar disorder and healthy volunteers. We found that patients had increased levels of both kynurenic acid and the pro-inflammatory cytokine IL-1β compared to healthy controls. In addition, patients had decreased levels of the pro-inflammatory cytokine IL-6. The presently observed increases in CSF IL-1β and kynurenic acid are in agreement with postmortem studies showing increased IL-1β mRNA expression and increased content of kynurenine (the immediate precursor of kynurenic acid) and TDO (one of the rate-limiting enzymes in kynurenic acid synthesis) in patients with bipolar disorder (Miller et al., 2006; 2008; Rao et al., 2010). Moreover, increased levels of CSF IL-1β have recently been demonstrated also in first episode patients with schizophrenia (Söderlund et al., 2009). In the present study, the mean CSF kynurenic acid content in patients with bipolar disorder was 1.71 nM, resulting in a 51% increase compared to the healthy controls. These results are similar to the previously reported increase of kynurenic acid in CSF of patients with schizophrenia, with mean levels ranging between 1.45 and 2.03 nM and an average increase of 53% compared to healthy controls (Erhardt et al., 2001a; Linderholm et al., 2010; Nilsson et al., 2005). Further, we found that kynurenic acid was age-correlated among patients, and an age-related increase of CSF kynurenic acid has also been reported in patients with schizophrenia (Nilsson et al., 2005). In contrast, the CSF kynurenic acid levels of healthy volunteers did not correlate with age in the present study. However, as our samples are limited by a rather narrow age range (24–51 years), we cannot rule out that brain kynurenic acid may increase with age in older healthy individuals.

Further, our results showed that CSF kynurenic acid was correlated with an increased risk of lifetime occurrence of psychotic symptoms and recent (during the year preceding CSF sampling) manic episodes in patients with bipolar disorder type I with odds ratios of 4.9 and 4.4, respectively. These findings further strengthen a pathophysiological role for kynurenic acid in bipolar disorder. It is tempting to speculate that increased brain kynurenic acid produces a hyperdopaminergic state that increases the risk of developing symptoms of mania and/or psychosis. This hypothesis
is reinforced by the strong correlation between the dopamine metabolite HVA and kynurenic acid observed in the CSF of patients with bipolar disorder (present results) and patients with schizophrenia (Nilsson et al., 2007).

In the present thesis, the CSF levels of cytokines and kynurenic acid were analyzed. Kynurenic acid has poor blood-brain crossing abilities, and CSF levels should therefore reflect brain levels rather than peripheral levels of this compound. This is also supported by unpublished data from our laboratory, showing a lack of correlation between plasma and CSF kynurenic acid levels in healthy volunteers. There is a delicate cross-talk between brain and peripheral immune systems, and cytokines do not readily cross the intact blood-brain barrier. Peripheral IL-1β and IL-6 has, however, been suggested to access the brain to some extent via saturable transport systems or via circumventricular organs (cf. Introduction). Circumventricular organs are small areas of the brain that partially lack a blood-brain barrier, allowing circulating molecules to infiltrate the tissue. However, anatomical barriers exist in the linings between the circumventricular organs and adjacent brain tissue as well as between the circumventricular organs and the CSF compartment (Krisch, 1978; 1986; Peruzzo et al., 2000; Rethelyi, 1984). Indeed, an autoradiography study has shown that peripheral IL-1 does not diffuse from the circumventricular organs into adjacent brain tissue and, further, that only a subset of vascular endothelial cells permit saturable transport IL-1 across the blood-brain barrier (Maness et al., 1998). In further support of a preferentially central origin of CSF cytokines, the present results showing increased IL-1β and decreased IL-6 clearly deviates from studies of serum/plasma cytokines in patients with bipolar disorder (Brietzke et al., 2009; Goldstein et al., 2009; Kauer-Sant'Anna et al., 2009; Ortiz-Dominguez et al., 2007). Further, correlations between peripheral and CSF cytokines are not evident in healthy individuals (Maier et al., 2005), and no correlations were found between the CSF and serum levels of IL-6 and IL-1β in a recent study with suicide attempters and healthy controls (Lindqvist et al., 2009). In addition, increased IL-1β mRNA has recently been reported in postmortem brain of patients with bipolar disorder (Rao et al., 2010), thus indicating that the increased CSF levels observed in the present study could arise from increased production of IL-1β in the brain.

The majority of patients enrolled in the present studies received on-going medication at the time of CSF sampling, and medication could therefore be a confounding factor in the present results. However, animal studies have shown that mood stabilizers such as valproic acid and lamotrigine do not affect brain kynurenic acid levels in rats when administered at clinically relevant concentrations (Kocki et al., 2006). In addition, chronic treatment with antipsychotic drugs reduce brain kynurenic acid levels in rats (Ceresoli-Borroni et al., 2006), and patients with schizophrenia with antipsychotic treatment at the time of death show a trend towards decreased cortical levels of kynurenic acid compared to patients without antipsychotic treatment at the time of death (Miller et al., 2006). Further, in the present thesis, patients taking antipsychotic drugs or lithium did not differ in their CSF kynurenic acid levels when compared with the rest of the patients. Both antipsychotic drugs and mood stabilizers have been shown to mediate anti-inflammatory effects. Thus, chlorpromazine reduces the production of TNF and IL-1β and increases anti-inflammatory IL-10 in mouse plasma/serum (Mengoazzi et al., 1994; Netea et al., 1995) and haloperidol decreases TNF and IL-1β in human monocytes.
kynurenine to 3-activity, an enzyme in the kynurenine pathway that catalyzes the conversion of kynurenine to 3-formylkynurenine. The rate-limiting step in kynurenine conversion to 3-formylkynurenine is dependent on the availability of kynurenine. The availability of kynurenine is thus determined by the enzymes IDO and TDO. Unpublished data from our laboratory show that IL-1β induces expression of both IDO and TDO and increases the production of kynurenine in cultured human astrocytes. However, increased density and intensity of TDO positive glial cells have been found in cortex of patients with bipolar disorder (Miller et al., 2006). Thus, induction of IDO/TDO by IL-1β might rationally explain the increased levels of kynurenine acid in these patients, however, no correlation between CSF levels of kynurenine acid and IL-1β was positively found. The availability of kynurenine is also determined by KMO activity, an enzyme in the kynurenine pathway that catalyzes the conversion of kynurenine to 3-HK. Blocking KMO thus leads to increased kynurenine acid synthesis.

(Kowalski et al., 2001; Moots et al., 1999). Further, chlorpromazine has been shown to reduce the secretion of IL-1β and IL-2 in LPS-stimulated rat glial cells (Labuzek et al., 2005). In the rat brain, lithium, valproic acid and lamotrigine mediate anti-inflammatory effects by reducing levels of COX-2 and PGE2 (Bosetti et al., 2002; Lee et al., 2008; Rao et al., 2007). Lithium downregulates NF-κB (a transcription factor involved in immune activation) in rat brain (Rao et al., 2007) and valproic acid inhibits TNF and IL-6 production, as well as NF-κB activation in human monocytes (Ichiyama et al., 2000). Downregulation of COX-2 and reduction of PGE2 levels have been suggested to be common mechanisms of action for mood stabilizers (Rao and Rapoport, 2009). Interestingly, selective COX-2 inhibitors (eg., celecoxib and parecoxib), are known to decrease the formation of brain kynurenic acid in rats (Schwieler et al., 2005; 2006; 2008), and improve symptoms of bipolar disorder, schizophrenia and major depression when given as adjunctive treatment (Akhoundzadeh et al., 2007; 2009; Muller et al., 2002; 2006; 2010; Nery et al., 2008). Notably, PGE2 has been shown to stimulate IL-6 release in human astroglioma cells and primary rat astrocytes (Fiebich et al., 2001). The presently observed reduction in CSF IL-6 levels in patients with bipolar disorder might therefore be an effect of on-going treatment with mood stabilizers. This notion is supported by the observation that IFN-α treatment in patients with hepatitis C, which is associated with the occurrence of mood symptoms and cognitive dysfunction, is accompanied by increased CSF levels of IL-6 (Raison et al., 2009). Further, increased CSF levels of IL-6 have been reported in suicide attempters compared to healthy controls (Lindqvist et al., 2009). Although on-going medication cannot be ruled out as an influencing factor, mood stabilizers and antipsychotic drugs generally display anti-inflammatory and COX-2 inhibitory properties. Hence, the present medication is unlikely to contribute to the increased levels of CSF IL-1β and kynurenic acid observed in the present thesis.

Although both CSF IL-1β and kynurenic acid levels were associated with recent manic symptoms, it is notable that all patients in these studies were euthymic at the time of sampling. If symptoms of mania are indeed caused by one or both of these compounds, the lack of affective symptoms in these euthymic patients is puzzling. However, it is possible that the levels of these biological markers of immune activation are even higher during acute mania than during a state of euthymia. Alternatively, having increased CSF IL-1β or kynurenic acid is not accompanied by manic symptoms in well-medicated individuals.

The reason for increased levels of kynurenic acid in patients with bipolar disorder is not known. The limiting factor in kynurenic acid synthesis is the availability of its precursor, kynurenine. Thus, the rate-limiting step in the production of kynurenic acid is the conversion of N-formylkynurenine to kynurenine by the enzymes IDO and TDO. Unpublished data from our laboratory show that IL-1β induces expression of both IDO and TDO and increases the production of kynurenic acid in cultured human astrocytes. Further, increased density and intensity of TDO positive glial cells have been found in cortex of patients with bipolar disorder (Miller et al., 2006). Thus, induction of IDO/TDO by IL-1β might rationally explain the increased levels of kynurenic acid in these patients, however, no correlation between CSF levels of kynurenic acid and IL-1β was presently found. The availability of kynurenine is also determined by KMO activity, an enzyme in the kynurenine pathway that catalyzes the conversion of kynurenine to 3-HK. Blocking KMO thus leads to increased kynurenic acid synthesis.
via shunting of the kynurenine catabolism towards kynurenic acid. In patients with schizophrenia, polymorphisms in the KMO gene are associated with high CSF levels of kynurenic acid (Holtze et al., 2011) and decreased KMO activity has been reported in brain regions with increased kynurenine and kynurenic acid levels (Sathyasaikumar et al., 2010). The activity of KMO in patients with bipolar disorder has thus far not been investigated. Altogether, the elevated CSF kynurenic acid content in patients with bipolar disorder may be causally related to their increased levels of IL-1β although no positive correlation between these immunomarkers was ascertained at the individual level.

Interestingly, accumulating data suggest that brain-derived cytokines might have additional functions that are separate from their role in mediating immune responses, and cytokines released from microglia, astrocytes, and even neurons in the brain are known to affect nerve growth, survival, proliferation and function. Astrocytes, which are able to produce cytokines such as IL-6 and IL-1β, are frequently co-localized with pre- and postsynaptic glutamatergic elements (cf. Introduction). It was recently demonstrated that Ca$^{2+}$ waves are produced in astrocytes in response to neurotransmitter stimulation, and trigger the release of various gliotransmitters and neuromodulators. Thus, astrocytes are able to actively modulate glutamate transmission in the brain. In rat hippocampal synapses, the IL-1RI is co-localized and bound to NMDA receptors (Gardoni et al., 2011), and stimulation with IL-1β causes a dose-dependent increase in NMDA receptor mediated Ca$^{2+}$ influx (Viviani et al., 2003). Further, both IL-1β and IL-6 are released during in vivo and in vitro LTP in rats (Schneider et al., 1998). While IL-1β was found to contribute to the production of LTP, IL-6 aided in the annihilation of LTP. Thus, IL-1β and IL-6, which are typically referred to as pro-inflammatory mediators, are also involved in hippocampal plasticity and memory processes in rodents. This new and unexpected role of cytokines as neuromodulating compounds with the ability to influence fundamental brain physiology might shed light on physiological and pathological brain processes that currently are poorly understood.

Another observation is that the CSF cytokine profile presently found in patients with bipolar disorder is not to be expected during infectious or autoimmune processes in the CNS, where a broader activation of pro-inflammatory mediators is commonly observed. IL-1β is a typical mediator of the innate immune response and a distinct and specific increase, as seen in patients with bipolar disorder, is therefore more consistent with an autoimmune inflammatory state. Whereas autoimmunity involves the adaptive immune response and the activation of lymphocytes directed against the own body, autoinflammation involves malfunctions in the non-specific, innate immune system. To our knowledge, any autoinflammatory disorders involving the CNS have not been described. In similarity with the recurrent nature of mood episodes in bipolar disorder, systemic autoinflammatory disorders (e.g., Familial Mediterranean Fever) are characterized by recurrent episodes of inflammation. The pathophysiology involves genetic deficits affecting the regulation of pro-inflammatory cytokines of the innate immune system and treatment regimes include inhibition of IL-1 signaling (Ombrello and Kastner, 2011). If patients with bipolar disorder or schizophrenia would benefit from treatment with IL-1 antagonists or have genetic deficits in the regulation of IL-1β signaling remains to be investigated.
Taken together, the results presented in this thesis show that patients with bipolar disorder have increased levels of CSF kynurenic acid and IL-1β that correlate to symptoms of mania and psychosis. We further show that prolonged elevation of brain kynurenic acid is accompanied by alterations in dopamine neurotransmission that are similar to clinical observations in patients with schizophrenia and bipolar disorder. Thus, these findings strengthen the hypothesis that kynurenic acid, possibly induced by increased levels of IL-1β, is functionally involved in producing a state of hyperdopaminergia that is believed to underlie symptoms of mania and/or psychosis. The present data, pointing towards a pathophysiological involvement of the pro-inflammatory mediator IL-1β and the astrocyte-derived neuromodulator kynurenic acid in psychiatric illnesses challenges the classical view of neurons as the key players in brain pathophysiology. Increasing the knowledge on neuron-glia interaction and shifting the attention towards pharmacological manipulation of glial neuroinflammatory signaling may present new treatment strategies and novel pharmacological targets for the psychotic disorders schizophrenia and bipolar disorder.
6 ACKNOWLEDGEMENTS

This work has been carried out at the Department of Physiology and Pharmacology, Karolinska Institutet, Stockholm, Sweden. I wish to express my sincere gratitude to all the people who have contributed to the completion of this thesis. In particular I want to thank:

My supervisor Dr. Sophie Erhardt, for her knowledge and skill in science, commitment to our work and constant support, for bringing me on conferences around the world and introducing me to the scientific community, for believing in me and letting me grow as a scientist.

Prof. Göran Engberg, for all his support, enthusiasm, laboratory technology skills, help with writing articles and this thesis, and for bringing a healthy amount of leisure into the group.

My co-supervisor Prof. Mikael Landén, for all his support, knowledge, enthusiasm, and help with writing articles.

My co-supervisor Dr. Camilla Svensson, for all her support and skill in science.

My co-supervisor Prof. Conny Nordin, who tragically passed away in July 2008, for his knowledge in science and for being an inspiring person.

My mentor Prof. Hans Jörnvall for his enthusiasm, knowledge, help with writing articles, for giving valuable advise, and for always taking the time to meet despite a busy schedule.

A huge thanks to all the fantastic members of the Engberg-Erhardt research group, for all the fun times, new experiences, and the life-long memories I will take with me:

Klas Linderholm, for his patience and help in the lab, for lightening up the long hours with laughs and fun, and for being a great travel companion.

Maria Holtze, my big sister in science and roomie, for all her help and support over the years, for being a great travel companion and friend, for following me on escapades, and for being the voice of reason. Btw, Skåne rules!

Magdalena Kegel, for all the fun times in the lab and elsewhere, for filling the lab with laughter, for her commitment, and for being a great person and co-worker.

Amanda Steen, for making group meetings a time to enjoy her delicious homemade cakes, for all the fun times, travels and parties, and for being a great friend.

Markus Larsson, for all his help with study II and this thesis, for excellent Mac support, for having worse tv habits than me, and for being a perfect roomie.

Lilly Schwieler, for help with writing articles, for sharing her knowledge in the lab, and for always lending a helping hand.
Acknowledgements

The rest of the members of the Engberg-Erhardt group: Ann-Chatrine Samuelsson, Kerstin Larsson, Alexandra Andersson, Elin Olsson, and Linda Nilsson-Todd, thanks for all your help and for the fun times we’ve shared!

All the co-authors of the papers and collaborators: Johan Söderlund for his knowledge and skill in science, for a great collaboration, all the good times and interesting talks, Carl Sellberg for a pleasant collaboration, his knowledge, patience, and help with statistics, Prof. Sharon Stone-Elander, Erik Samén, Li Lu, Martina Wennberg, Martin Samuelsson, Peter Sætre, Leif Lindström, Erik Jönsson, Lilian Walther-Jallow and Christian Johansson for a fruitful and pleasant collaboration.

Jan Kowalski, for generously offering support and being “nödens ängel”.

Joshua Gregory, for critical reading of this thesis and helping with the English language.

Jon Lampa, for interesting discussions and valuable help.

Prof. Stefan Eriksson, head of the Department of Physiology and Pharmacology, for providing good working conditions.

All the patients and healthy volunteers who made this work possible.

Helena for her important contribution to science.

All the former and present Fyfa PhD students, friends and co-workers, especially Åsa Key, Björn, Monica, Aki, Torun, Calle, Pixie, Lotta, Mickis, Carina, Jacomijn, Anna, Frank, Louise, Elinor, Lars, Ebba, Gustaf and Daniella, thanks for all your help and support, and for all the fun times together!

old and new SU, KI, and KTH friends, especially Mike, Marianna, Petra, Daniel, Linnea, Emma, Nico, Essam, Olle, Daniel, Ida, Johan, Sofia, Christine, Lisa, Henrik, Stefan, Roland and Jennifer.

Charlotte Mattsson, my first supervisor and friend, for her outstanding help and support with everything, for all the fun times at MF and Gröna Villan, Harry Potter marathons, cray fish and midsummer parties, Wee nights, and for being a wonderful person and a great friend.

My dear friends Katarina Eisleitner and Hanna Nilsson, for being a part of my life. Thanks for all the girl time, dinners, long nights, travels, for sharing the delights and troubles, big and small things in life with me! You are two “one of a kind”, love you both!

My Haninge childhood friends: Lise-Lotte Steen, Cicci Risfjell, Anna Andersson, Melissa Söderback and Linda Johansson, thank you for all the memorable and fun times!
My mom and dad, for loving me like a daughter but never treating me like a girl, for always believing in me and letting me go my own way in life. Thank you for everything. Love you so much!

My brother, for always looking out for me and being my friend for as long as I can remember, for always including me, for teaching me how to get my hands dirty, for being a truly great guy and for being MY big brother for life!

Noah, il mio mostro preferito! Thank you for being so fantastic, for seeing me for who I am and for being the warmest and most real person I know 💖
7 REFERENCES


Andén, NE, Carlsson, A, Dahlström, A, Fuxe, K, Hillarp, NA, Larsson, K (1964)
Demonstration and Mapping out of Nigro-Neostriatal Dopamine Neurons. Life Sci, 3: 523-530


Brown, AS, Schaefer, CA, Quesenberry, CP, Jr., Liu, L, Babulas, VP, Susser, ES (2005) Maternal exposure to toxoplasmosis and risk of...


Carlsson, A, Lindqvist, M (1963) Effect of chlorpromazine or haloperidol on formation of 3methoxytyramine and normetanephrine in mouse brain Acta Pharmacol Toxicol (Copenh), 20: 140-144


Bunney, BS, Aghajanian, GK (1978) d-Amphetamine-induced depression of central dopamine neurons: evidence for mediation by both autoreceptors and a striato-nigral feedback pathway. Naunyn Schmiedebergs Arch Pharmacol, 304(3): 255-261


Sara Olsson
References


inflammation to sickness and depression: when the immune system subjugates the brain. Nat Rev Neurosci, 9(1): 46-56


References


Gonon, FG (1988) Nonlinear relationship between impulse flow and dopamine released by rat midbrain dopaminergic neurons as

Goodall, E (1932) The existing cause of certain states, at present classified under 'schizophrenia' by psychiatrists may be infection. J Ment Sci, 78: 746


Green, AL, el Hait, MA (1978) Inhibition of mouse brain monoamine oxidase by (+)-amphetamine in vivo. J Pharm Pharmacol, 30(4): 262-263


Grotta, J, Clark, W, Coull, B, Pettigrew, LC, Mackay, B, Goldstein, LB, Meissner, I, Murphy, D, LaRue, L (1995) Safety and tolerability of the glutamate antagonist CGS 19755 (Selfotel) in patients with acute ischemic stroke. Results of a phase IIa randomized trial. Stroke, 26(4): 602-605


Jentsch, JD, Tran, A, Le, D, Youngren, KD, Roth, RH (1997b) Subchronic phencyclidine administration reduces mesoprefrontal dopamine utilization and impairs prefrontal cortical-dependent cognition in the rat. Neuropsychopharmacology, 17(2): 92-99


Jentsch, JD, Taylor, JR, Roth, RH (1998b) Subchronic phencyclidine administration increases mesolimbic dopaminergic system responsivity and augments stress- and psychostimulant-induced hyperlocomotion. Neuropsychopharmacology, 19(2): 105-113


References


Knable, MB, Weinberger, DR (1997) Dopamine, the prefrontal cortex and schizophrenia. J Psychopharmacol, 11(2): 123-131


assessed by microdialysis and rapid electrochemistry. Neuroscience, 169(4): 1848-1859


References


Leeson, VC, Barnes, TR, Hutton, SB, Ron, MA, Joyce, EM (2009a) IQ as a predictor of functional outcome in schizophrenia: a longitudinal, four-year study of first-episode psychosis. Schizophr Res, 107(1): 55-60


86


References


Passera, E, Campanini, B, Rossi, F, Casazza, V, Rizzi, M, Pellicciari, R, Mozzarelli, A (2011) Human kynurenine aminotransferase II-
reactivity with substrates and inhibitors. FEBS J, 278(11): 1882-1900


Rice, SR, Niu, N, Berman, DB, Heston, LL, Sobell, JL (2001) Identification of single nucleotide polymorphisms (SNPs) and other sequence changes and estimation of nucleotide diversity in coding and flanking regions of the NMDAR1 receptor gene in schizophrenic patients. Molecular Psychiatry, 6(3): 274-284


thalamus is a key target for brain dopamine. J Neurosci, 25(26): 6076-6083


Schwieler, L, Linderholm, KR, Nilsson-Todd, LK, Erhardt, S, Engberg, G (2008) Clozapine interacts with the glycine site of the NMDA receptor: electrophysiological studies of
dopamine neurons in the rat ventral tegmental area. Life Sci, 83(5-6): 170-175


Speciale, C, Wu, HQ, Cini, M, Marconi, M, Varasi, M, Schwarz, R (1996) (R,S)-3,4-dichlorobenzoylalanine (FCE 28833A) causes a large and persistent increase in brain kynurenic
References

tonic acid levels in rats. Eur J Pharmacol, 315(3): 263-267


Tsukada, H, Nishiyama, S, Fukumoto, D, Sato, K, Kakiuchi, T, Domino, EF (2005) Chronic NMDA antagonism impairs working memory, decreases extracellular dopamine, and increases D1 receptor binding in prefrontal cortex of conscious monkeys. Neuropsychopharmacology, 30(10): 1861-1869


Wohl, M, Gorwood, P (2007) Paternal ages below or above 35 years old are associated with a different risk of schizophrenia in the offspring. Eur Psychiatry, 22(1): 22-26


