IMBALANCED KYNUREНИNE PATHWAY IN SCHIZOPHRENIA AND DEPRESSION - IMMUNOLOGICAL AND GENETIC ASPECTS

Xicong Liu

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Imbalanced Kynurenine Pathway in Schizophrenia and Depression - Immunological and Genetic Aspects
THESIS FOR DOCTORAL DEGREE (Ph.D.)

By

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To my family
ABSTRACT

Kynurenic acid (KYNA), an end metabolite of the kynurenine pathway along tryptophan degradation, has gained increasing interest in the pathophysiology of psychiatric disorders. Elevated levels of KYNA have been found in the cerebrospinal fluid (CSF) and in the postmortem brain of patients with schizophrenia and bipolar disorder with psychotic symptoms. In contrast, reduced levels of KYNA are associated with depressive symptoms. Rapidly emerging evidence points to the involvement of brain immune activation in psychiatric disorders and to the kynurenine pathway as a causal link between brain immune activation and psychiatric disorders. The aim of present thesis was to investigate the role of the kynurenine pathway in the pathophysiology of schizophrenia and depression in experimental settings.

The results show that an infection with neurotropic influenza A virus or a transient elevation of brain KYNA levels in neonatal mice enhanced the sensitivity of d-amphetamine-induced increase in locomotor activity in adulthood. Neonatal elevation of KYNA also impaired prepulse inhibition and working memory in adulthood. These long-lasting behavioral alterations suggest that the kynurenine pathway as a causal link between early-life infection and the development of neuropsychiatric disturbances in adulthood.

Reduced KYNA levels were detected in the prefrontal cortex, but not in the frontal cortex, hippocampus, striatum or cerebellum, of Flinders Sensitive Line (FSL) rats, an animal model of depression, compared with their controls, the Flinders Resistant Line (FRL) rats.

Inhibition of Kynurenine 3-monooxygenase (KMO) shunts the kynurenine pathway towards enhanced synthesis of KYNA. Mice with a targeted deletion of KMO exhibited impairments in contextual memory and social interaction, potentiated horizontal activity following d-amphetamine-induced increase in locomotor activity as well as increased anxiety-like behaviors. In addition, genome-wide differential gene expression analyses identified alterations regarding schizophrenia- and psychosis-related genes in these mice.

Kynurenine aminotransferase (KAT) II is identified as the main enzyme responsible for most of brain KYNA production. Nevertheless, KAT II KO mice receiving kynurenine injection or repeated injections of Lipopolysaccharide (LPS) exhibited increased concentrations of brain KYNA.

Taken together, these results give strong experimental support for the connection between immune activation and KYNA in schizophrenia and suggest that low brain KYNA could be of importance for the depressive-like behaviors observed in FSL rats.
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*Contributed equally*
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<tr>
<td>3-HK</td>
<td>3-hydroxykynurenine</td>
</tr>
<tr>
<td>AADAT</td>
<td>Aminoadipate aminotransferase</td>
</tr>
<tr>
<td>AMPA</td>
<td>α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid</td>
</tr>
<tr>
<td>ANOVA</td>
<td>Analysis of variance</td>
</tr>
<tr>
<td>ASAT</td>
<td>Mitochondrial aspartate aminotransferase</td>
</tr>
<tr>
<td>BBB</td>
<td>Blood-brain barrier</td>
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<tr>
<td>CCBL</td>
<td>Cysteine conjugate beta-lyas</td>
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<tr>
<td>CNS</td>
<td>Central nerves system</td>
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<tr>
<td>COX</td>
<td>Cyclo-oxygenase</td>
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<tr>
<td>CSF</td>
<td>Cerebrospinal fluid</td>
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<tr>
<td>DEGs</td>
<td>Differentially expressed genes</td>
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<tr>
<td>DFP</td>
<td>Diisopropyl fluorophosphates</td>
</tr>
<tr>
<td>DISC</td>
<td>Disrupted-in-schizophrenia</td>
</tr>
<tr>
<td>FRL</td>
<td>Flinders Resistant Line</td>
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<tr>
<td>FSL</td>
<td>Flinders Sensitive Line</td>
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<tr>
<td>GOT</td>
<td>Glutamic-oxaloacetic transaminase</td>
</tr>
<tr>
<td>GTK</td>
<td>Glutamine transaminase K</td>
</tr>
<tr>
<td>GWAS</td>
<td>Genome-wide association studies</td>
</tr>
<tr>
<td>HPA</td>
<td>Hypothalamic-pituitary-adrenal</td>
</tr>
<tr>
<td>HPLC</td>
<td>High performance liquid chromatography</td>
</tr>
<tr>
<td>HSV-2</td>
<td>Herpes simplex virus type 2</td>
</tr>
<tr>
<td>i.e.</td>
<td>That is (id est lat.)</td>
</tr>
<tr>
<td>i.p.</td>
<td>Intraperitonatally</td>
</tr>
<tr>
<td>IC&lt;sub&gt;50&lt;/sub&gt;</td>
<td>Half maximal inhibitory concentration</td>
</tr>
<tr>
<td>IDO</td>
<td>Indoleamine 2,3-dioxygenase</td>
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<tr>
<td>IFN</td>
<td>Interferon</td>
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<tr>
<td>IL</td>
<td>Interleukin</td>
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<td>KAT</td>
<td>Kynurenine aminotransferases</td>
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<td>Km</td>
<td>Michaelis-Menten constant</td>
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<td>KMO</td>
<td>Kynurenine 3-monooxygenase</td>
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<tr>
<td>Acronym</td>
<td>Full Form</td>
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<td>---------</td>
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<tr>
<td>KO</td>
<td>Knockout</td>
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<tr>
<td>KYNA</td>
<td>Kynurenic acid</td>
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<tr>
<td>KYNU</td>
<td>Kynureninase</td>
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<tr>
<td>LPS</td>
<td>Lipopolysaccharide</td>
</tr>
<tr>
<td>MDD</td>
<td>Major depressive disorder</td>
</tr>
<tr>
<td>NAD⁺</td>
<td>Nicotinamide adenine dinucleotide</td>
</tr>
<tr>
<td>NMDA</td>
<td>N-methyl-D-aspartate</td>
</tr>
<tr>
<td>PAP</td>
<td>Passive avoidance paradigm</td>
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<tr>
<td>PBS</td>
<td>Phosphate buffered saline</td>
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<tr>
<td>PCP</td>
<td>Phencyclidine</td>
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<tr>
<td>PET</td>
<td>Positron Emission Tomography</td>
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<tr>
<td>PIC</td>
<td>Picolinic acid</td>
</tr>
<tr>
<td>PND</td>
<td>Postnatal day</td>
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<td>PPI</td>
<td>Prepulse inhibition</td>
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<tr>
<td>QUIN</td>
<td>Quinolinic acid</td>
</tr>
<tr>
<td>RIN</td>
<td>RNA Integrity Number</td>
</tr>
<tr>
<td>RM</td>
<td>Repeated measure</td>
</tr>
<tr>
<td>TDO</td>
<td>Tryptophan 2,3-dioxygenase</td>
</tr>
<tr>
<td>TLR</td>
<td>Toll-like receptors</td>
</tr>
<tr>
<td>TNF</td>
<td>Tumor necrosis factor</td>
</tr>
<tr>
<td>α7nACh</td>
<td>α7 nicotinic acetylcholine</td>
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</table>
1 INTRODUCTION

1.1 Schizophrenia

The history of schizophrenia can be retrospected as early as 1550BC as the oldest documentation about an illness like schizophrenia is found in Ebers Papyrus from ancient Egypt (Burton, 2012). Symptoms similar to those manifested in schizophrenia are also described in the Bible. In the late 19th century, the German psychiatrist Emil Kraepelin integrated the pieces of various clinical observations of mental disorders into a single category termed “dementia praecox” (Kraepelin, 1896). Later, the Swiss psychiatrist Eugen Bleuler developed Kraepelin’s theory and replaced the term with “schizophrenia” in 1911 (Bleuler, 1911).

1.1.1 Symptoms of schizophrenia

Patients with schizophrenia often have distorted perception of reality caused by a variety of symptoms. The symptoms are generally classified into three distinct categories (Andreasen and Olsen, 1982):

- **Positive symptoms**, also known as psychotic symptoms, refer to features not seen in healthy population but present in patients, such as hallucinations, delusions as well as disorganized thought processes.

- **Negative symptoms** are characterized by withdrawal or lack of function that is normally present in healthy people, such as anhedonia, social withdrawal, blunted affect and loss of motivation.

- **Cognitive dysfunctions** refer to a wide range of features regarding difficulties in problem solving, impairments in working memory and attention.

1.1.2 Prevalence and onset of schizophrenia

Schizophrenia has a global lifetime prevalence of approximately 0.40-0.87% (McGrath et al., 2008; Perälä et al., 2007; Saha et al., 2005). Schizophrenia is often accompanied with high suicide rates, approximately 5-10% higher than general population (Black and Fisher, 1992; Meltzer, 2002; Palmer et al., 2005). The average life expectancy in woman and man with
Schizophrenia is 12 and 15 years shorter, respectively, than the rest of the population (Crump et al., 2013).

The onset of schizophrenia generally emerges in late adolescence or early adulthood. Since adolescence is a critical period for the development of the brain, the pathogenesis of schizophrenia may be embedded during early development (Paus, 2005; Paus et al., 2008; Welham et al., 2009). The neurodevelopmental theory of schizophrenia was suggested in the 1980s (Lewis, 1989; Lyon et al., 1989; Murray and Lewis, 1988; Weinberger, 1987) and proposed that early brain insults, caused by environmental or genetic risk factors, affect brain development and lead to pathological processes of schizophrenia (Rapoport et al., 2005).

1.1.3 Risk factors of schizophrenia

1.1.3.1 Environmental risk factors
Environmental risk factors related to prenatal, neonatal and early life have been highlighted by several studies pointing to the etiology of schizophrenia. In particular, increased risk of schizophrenia was found in subjects born in the winter or in the beginning of spring (Davies et al., 2003; Torrey et al., 1997) as well as in individuals born in urban area (Marcelis et al., 1999; Mortensen et al., 1999). Moreover, prenatal stress or early life trauma can also be risk factors for the development of the disease (Huttunen and Niskanen, 1978; Khashan et al., 2008; Morgan and Fisher, 2007; van Os and Selten, 1998). Furthermore, prenatal malnutrition regarding folate, iron and vitamin D are correlated with the development of the disease (Brown et al., 1996; Insel et al., 2008; McGrath, 1999; Rosso, 1990). In addition, studies have provided evidences for the association between maternal and postnatal infections, induced for example by influenza, Toxoplasma gondii and herpes simplex virus type 2 (HSV-2), and schizophrenia (Brown and Derkits, 2010; Dalman et al., 2008; Meyer, 2013). Therefore, it seems that this is not a pathogen-specific association; instead it sheds light upon the mediation of upregulated expression of inflammatory cytokines (Brown et al., 2004; Buka et al., 2001), suggesting an involvement of immunological factors in schizophrenia.

1.1.3.2 Genetic risk factors
For decades, family history of schizophrenia has been identified as the most predominant risk factor for the disease. When both parents are affected by schizophrenia, there is a relative risk of 89% for their child to develop the disease (Lichtenstein et al., 2006). Furthermore, twins studies have provided evidence showing that genetic factors account for more than 80% of the risk for the development of schizophrenia (Cannon et al., 1998; Cardno et al., 1999;
Sullivan et al., 2003). However, the complexity of the disease makes the search for a causal gene a hard and tortuous journey. In all probability, due to cooperative effects of multiple vulnerable genes that interact with environmental risk factors (Harrison and Owen, 2003).

1.1.4 Hypotheses of schizophrenia

1.1.4.1 The dopamine hypothesis of schizophrenia

The dopamine hypothesis has been one of the main theories of the pathophysiology of schizophrenia, proposing that dopaminergic hyperactivity is responsible for the positive symptoms observed in the disorder (Carlsson and Lindqvist, 1963). The hypothesis originates from findings that antipsychotic drugs have the potency to ameliorate the symptoms of schizophrenia by blocking dopamine D$_2$ receptors (Creese et al., 1976; Seeman and Lee, 1975; Seeman et al., 1976). In addition, dopamine enhancing drugs, such as amphetamine, can deteriorate the symptoms in patients with schizophrenia (Angrist et al., 1974) and induce a state of psychosis in healthy people (Snyder, 1973). Nevertheless, negative symptoms and cognitive impairments of schizophrenia have drawn more attentions for many years, and their treatment resistance to antipsychotic drugs led to a revision of the dopamine hypothesis (Breier, 1999; King, 1998). The revised dopamine hypothesis proposes an imbalance of dopaminergic transmission in schizophrenia involving hyperactivation of the subcortical mesolimbic dopamine pathway, which leads to positive symptoms, and a hypoactivation of the mesocortical dopamine projection to the prefrontal cortex resulting in negative symptoms and cognitive dysfunctions (Abi-Dargham and Laruelle, 2005; Davis et al., 1991; Goldman-Rakic et al., 2000).

1.1.4.2 The glutamate hypothesis of schizophrenia

The glutamate deficiency theory of schizophrenia was initially generated from a report suggesting low glutamate levels in the cerebrospinal fluid (CSF) of patients with schizophrenia (Kim et al., 1980). Around the same time, phencyclidine (PCP), known as an anesthetic, was found to induce schizophrenia-like symptoms in healthy volunteers and to exacerbate existing symptoms in patients with schizophrenia (Allen and Young, 1978; Itil et al., 1967; Luby et al., 1959). Later, PCP was characterized as a non-competitive N-methyl-D-aspartate (NMDA) receptor antagonist and found to reduce glutamate neurotransmission (Anis et al., 1983; Lodge and Anis, 1982). Moreover, ketamine, another non-competitive NMDA receptor antagonist, is shown to aggravate symptoms in patients with schizophrenia (Lahti et al., 2001; Malhotra et al., 1997) and mimic all spectra of symptoms in healthy individuals (Krystal et al., 1994). Furthermore, several studies have implied that competitive
NMDA receptor antagonists as well as compounds that block the glycine site of the NMDA receptor could evoke the symptoms of schizophrenia in human (Albers et al., 1999; Grotta et al., 1995; Kristensen et al., 1992; Yenari et al., 1998), which further give support to the glutamate deficiency theory.

The dopamine hypothesis and the glutamate deficiency theory are not totally irrelevant hypotheses. Several studies have confirmed that blockade of NMDA receptor leads to an increased dopamine activity in the midbrain of rodents (Erhardt and Engberg, 2002; French, 1994; French et al., 1993; Linderholm et al., 2007; Schwieler et al., 2004). Besides, administration of ketamine in healthy volunteers induces an increase of dopamine release and potentiates the amphetamine-induced dopamine response in striatum (Breier et al., 1998; Kegeles et al., 2000; Vollenweider et al., 2000). Altogether, these two theories support each other by implicating that NMDA receptor hypofunction induces an imbalance of dopamine neurotransmission in schizophrenia (Svensson, 2000).

1.1.4.3 The Kynurenic acid hypothesis of schizophrenia

In recent years, the kynurenic acid (KYNA) hypothesis of schizophrenia has drawn more and more attention. The hypothesis is constructed on the fact that KYNA is an endogenous antagonist of the NMDA receptor as well as of the α7 nicotinic acetylcholine (α7nACh) receptor in the brain (Hilmas et al., 2001; Stone, 1993). Several studies have shown that patients with schizophrenia display elevated levels of KYNA in the CSF (Erhardt et al., 2001a; Linderholm et al., 2012; Nilsson et al., 2005) as well as in the prefrontal cortex of postmortem brain (Sathyasaikumar et al., 2011; Schwarcz et al., 2001). In line with these studies, it has also been shown that kynurenine, the precursor of KYNA, is increased in the CSF and postmortem brain of patients (Linderholm et al., 2012; Miller et al., 2006).

Fluctuations in endogenous KYNA levels mediate alterations of dopamine neurotransmission. Thus, it has been shown that pharmacological elevation of KYNA increases firing of rat midbrain dopamine neurons (Erhardt and Engberg, 2002; Erhardt et al., 2001b; Linderholm et al., 2007; Nilsson et al., 2006), and reduced concentration of KYNA decreases the activity (Linderholm et al., 2016; Schwieler et al., 2006; Schwieler et al., 2008). Furthermore, subchronical elevation of KYNA distinctly enhanced the dopaminergic response to an amphetamine challenge (Olsson et al., 2009). This finding is in consonance with clinical observations of patients with schizophrenia showing hyper-dopaminergic response to amphetamine compared to healthy volunteers (Abi-Dargham et al., 1998; Laruelle, 1998; Laruelle and Abi-Dargham, 1999). Indeed, increased midbrain dopamine firing contributes to
the excess of subcortical dopamine, which is involved in the generation of positive symptoms in schizophrenia (Davis et al., 1991; Jentsch and Roth, 1999).

The KYNA hypothesis is further supported by a large amount of studies showing the association of NMDA and α7nACh receptors with cognitive functions (Timofeeva and Levin, 2011). Cognitive deficits, such as impairments in working memory, speed of processing, attention, learning and memory, problem solving are considered as core features in schizophrenia (Nuechterlein et al., 2004). The hypothesis has been tested in several behavioral experimental settings to understand how fluctuations in KYNA levels affect cognitive function. It has been shown that elevated levels of KYNA induce impairments in contextual processing (Alexander et al., 2012; Chess and Bucci, 2006), spatial working memory (Chess et al., 2007; Pocivavsek et al., 2011) as well as contextual memory (Chess et al., 2009), while lower levels of KYNA improve cognitive performance (Kozak et al., 2014; Potter et al., 2010). These results are in line with a recent study showing that elevated levels of KYNA are associated with cognitive impairments in patients with bipolar disorder (Sellgren et al., 2015). In addition, increased levels of KYNA disrupt prepulse inhibition (PPI) in the rat (Erhardt et al., 2004). PPI is associated with sensorimotor gating and reflects the ability of the brain to filter out abundant information. Indeed, deficits in PPI and sensorimotor gating are a core deficit of patients with schizophrenia (Braff and Geyer, 1990).

Taken together, the kynurenic acid hypothesis of schizophrenia covers the whole spectra of symptoms of schizophrenia and links the dopamine and glutamate hypotheses to explain the possible underlying pathophysiology mechanisms of the disease.

1.2 Depression

Depression is a complex, multifactorial, heterogeneous and recurrent mental disorder with estimated lifetime prevalence from 11.1% to 14.6% among different counties (Bromet et al., 2011). It was ranked as the fourth leading cause of disability worldwide (Murray and Lopez, 1996) by World Health Organization and is projected as the second by 2020 (Murray and Lopez, 1997). Patients suffering from major depressive disorder (MDD) often experience multiple recurrences of the disease. Approximate two-thirds of the patients developed at least one recurrence (Solomon et al., 2000). Patients with depression often present persistent low mood and anhedonia accompanied by physical and mental alterations causing impairments in social, occupational and other capacity of functioning.
1.3 The kynurenine pathway

KYNA, when discovered in dog urine by the German chemist Justus von Liebig in 1853 (Liebig, 1853), was the first identified member of the kynurenine pathway. Half a century later, KYNA was recognized as a metabolite of tryptophan (Alexander, 1904), an essential amino acid required for the biosynthesis of proteins. Subsequently, with the identification of other metabolites of tryptophan degradation towards kynurenine, this pathway was termed the “kynurenine pathway” (Beadle et al., 1947). The majority of tryptophan is metabolized through the kynurenine pathway. Only less than 5% of tryptophan goes into the serotonin pathway (Gal and Sherman, 1980). Therefore, the kynurenine pathway is the main route of tryptophan degradations, accounting for approximate 90-95% of tryptophan in most mammalian tissues (Gal and Sherman, 1980; Leklem, 1971).

1.3.1 Kynurenic acid

KYNA is an endogenous antagonist acting on a wide range of glutamate receptors. At low concentrations, it blocks the strychnine-insensitive glycine site of the NMDA receptor \([\text{IC}_{50} \approx 8-15 \ \mu M]\) (Birch et al., 1988; Kessler et al., 1989; Parsons et al., 1997) and the \(\alpha_7\nACh\) receptor \([\text{IC}_{50} \approx 7 \ \mu M]\) (Hilmas et al., 2001; Stone, 2007). At higher concentrations, it also blocks the glutamate recognition site of the NMDA receptor \([\text{IC}_{50} \approx 200-500 \mu M]\) (Kessler et al., 1989). At millimolar concentrations, KYNA competitively antagonizes on the \(\alpha\)-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA) receptor as well as the kainate receptor (Kessler et al., 1989). Additional targeting receptors of KYNA have been identified during the last couple of years. Thus, studies have suggested that KYNA acts as an agonist of the former orphan G protein-coupled receptor GPR35 (Berlinguer-Palmini et al., 2013; Resta et al., 2016) and as an efficient agonist of the aryl hydrocarbon receptor. Stimulation of the aryl hydrocarbon receptor with KYNA in the presence of interleukin (IL)-1β synergistically induces IL-6 expression. (DiNatale et al., 2010).

1.3.2 Enzymatic steps

The initial and rate-limiting step of the kynurenine pathway (Figure 1) is the oxidative opening of the indole ring of tryptophan to produce N-formyl kynurenine. Two enzymes are responsible for this process; indoleamine 2,3-dioxygenase (IDO1) and/or tryptophan 2,3-dioxygenase (TDO2). Subsequently, N-formyl kynurenine is rapidly hydrolyzed to kynurenine, the pivotal metabolite, by kynurenine formamidase. Kynurenine is able to pass
the blood-brain barrier (BBB) and is further catabolized to three different molecules: 1) Kynurenine aminotransferases (KAT I, KAT II, KAT III and KAT IV), which catabolize kynurenine towards KYNA 2) kynurenine 3-monoxygenase (KMO), which converts the degradation of kynurenine to 3-hydroxykynurenine (3-HK) and 3) kynureninase (KYNU), which forms anthranilic acid. KYNA is an end product, whereas 3-HK and anthranilic acid are subsequently metabolized by KYNU or a nonspecific oxidation to form 3-hydroxyanthranilic acid and then further converted to quinolinic acid (QUIN), which, as opposed to KYNA, is an agonist of the NMDA receptor (Stone and Perkins, 1981). The final metabolism along the QUIN branch leads to the production of NAD⁺, which is an oxidized form of nicotinamide adenine dinucleotide (Moroni, 1999; Stone, 1993).

Figure 1. The kynurenine pathway
Enzymes involved in the kynurenine pathway have been found both in the central nervous system (CNS) and in the periphery. After the transportation of kynurenine from circulation to the CNS, it is taken up by glial cells (Speciale and Schwarcz, 1990). Notably, KAT enzymes, which convert kynurenine to KYNA, are primarily expressed in astrocytes (Guillemin et al., 2001b), however, KMO and KYNU enzymes, which participate in the 3-HK synthesis and further downstream production of QUIN, are mainly expressed in microglia (Guillemin et al., 2003). Thus, the two different branches of the kynurenine pathway are spatially segregated in the brain.

KAT enzymes have been identified with four homologues (Han et al., 2010a): KAT I/glutamine transaminase K (GTK)/cysteine conjugate beta-lyas (CCBL)1, KAT II/aminoadipate aminotransferase (AADAT), KAT III/CCBL2, KAT IV/glutamic-oxaloacetic transaminase (GOT)2/mitochondrial aspartate aminotransferase (ASAT). All these four enzymes are considered to be involved in the formation of KYNA in the brain (Guidetti et al., 2007; Guidetti et al., 1997; Okuno et al., 1991; Yu et al., 2006), however, due to different characteristics of the four isoforms such as substrate specificity, optimum pH and temperature, KAT II enzyme stands out to be the main biosynthesis enzyme for KYNA under physiological conditions (Han et al., 2010b; Schmidt et al., 1993).

1.3.3 Regulation of the kynurenine pathway

The KAT II enzyme has a Michaelis Menten constant ($K_m$) in millimolar range, suggesting that the enzyme is far from saturated under normal physiological conditions (Okuno et al., 1991). Thus, the production of KYNA is largely dependent on the availability of its precursor, kynurenine, and indeed, systematic administration of kynurenine increases the levels of KYNA in brains in both rats and primates (Jauch et al., 1993; Swartz et al., 1990; Wu et al., 1992).

The activity of the kynurenine pathway is regulated by several distinct mechanisms. Intracellular concentration of amino acid such as glutamine and phenylalanine, which are the competitive substrates of KAT I and KAT II, have been demonstrated to control the formation of KYNA in the brain (Chang et al., 1997). KMO is the enzyme converting the degradation of kynurenine towards QUIN branch in the kynurenine pathway and pharmacological blockade of KMO leads to shunting of the metabolism of kynurenine towards KYNA (Rover et al., 1997; Russi et al., 1992; Speciale et al., 1996). Moreover, cyclo-oxygenase (COX)-1 inhibition with indometacine or diclofenac indirectly increases KYNA levels in the brain (Edwards et al., 2000; Schwieler et al., 2005; Schwieler et al.,
2006), whereas, systematic administration of parecoxib or meloxicam, which act as COX-2 inhibitors, decreases KYNA concentrations in the brain (Schwieler et al., 2005; Schwieler et al., 2006). Furthermore, KYNA is actively extruded from the CNS by organic anion transporters (Nemeth et al., 2005; Uwai et al., 2012). To avoid its extrusion, probenecid is used to block organic anion transporter activity (Cunningham et al., 1981; Moroni et al., 1988). Notably, pro-inflammatory cytokines are also able to induce the rate-limiting enzymes, IDO1 (Carlin et al., 1987; Yoshida et al., 1986) and TDO2 (Miller et al., 2006; Sellgren et al., 2015), which lead to increased levels of kynurenine and further induction of the entire pathway. Thus, this evidence implies that immune activation is involved in the regulation of the kynurenine pathway.

1.3.4 Regulation of the kynurenine pathway following immune activation

Cytokines are released from innate immune cells under immune response. The principal cytokine to induce IDO1 is interferon (IFN)-γ (Pemberton et al., 1997), which has been shown to increase IDO1 transcription in many cell types such as macrophages, microglia, dendritic cells, epithelial cells, smooth muscle cells and several tumor cell lines (Alberati-Giani et al., 1996; Chiarugi et al., 2001; Cuffy et al., 2007; Jeong et al., 2009; Mailankot and Nagaraj, 2010; Takikawa, 2005). Other cytokines including IFN-α, IFN-β and tumor necrosis factor (TNF)-α have also the capacity to induce IDO1, but to a less degree than IFN-γ (Guillemin et al., 2001a; Pemberton et al., 1997). Recent studies have shown that the other rate-limiting enzyme TDO2 is induced by IL-1β (Sellgren et al., 2015; Urata et al., 2014). Moreover, IFN-γ has been shown to induce KMO expression as well as its activity (Alberati-Giani et al., 1996; Chiarugi et al., 2001). Following systemic inflammatory stimulation by Lipopolysaccharide (LPS), KMO expression is enhanced together with increased levels of TNF-α and IL-6 in rat brains (Connor et al., 2008).

1.3.5 The kynurenine pathway in schizophrenia and depression

As described in the “kynurenic acid hypothesis of schizophrenia” section, KYNA levels are elevated in both CSF and postmortem brains of patients with schizophrenia (Erhardt et al., 2001a; Schwarcz et al., 2001). Other metabolites and enzymes involved in the kynurenine pathway have also been measured. Importantly, a reduction of KMO gene expression and enzyme activity has been discovered in patients with schizophrenia and bipolar disorder (Lavebratt et al., 2014; Sathyasaikumar et al., 2011; Wonodi et al., 2011). Thus, the reduced activity in KMO shunts the metabolism of kynurenine towards KYNA (Sathyasaikumar et al.,
2011). Interestingly, studies investigating rate-limiting enzymes of the kynurenine pathway revealed that only TDO2 expression, but not IDO1, is upregulated in brains of patients with schizophrenia and bipolar disorder with psychosis (Miller et al., 2004; Miller et al., 2006).

Participation of the kynurenine pathway in depression was suggested in 1970’s (Lapin, 1973), and implied a connection between tryptophan, kynurenine and depression. However, due to the achievement of antidepressants using serotonin reuptake inhibitors, the focus of depression research shifted to serotonin. The involvement of the kynurenine pathway in depression is now gaining increased attention and for instance IDO1 activation is suggested to play a critical role in the induction of depressive symptoms. Pro-inflammatory cytokines, such as IFN-α, stimulate IDO1 and further induce depressive symptoms in patients with hepatitis C and malignant melanoma (Capuron et al., 2003; Raison et al., 2010). Moreover, animal studies have demonstrated that bacterial infection or upregulation of IDO1 by IFN-γ and TNF-α leads to long-lasting depressive-like behaviors (O'Connor et al., 2009a; O'Connor et al., 2009b). The elevation of downstream neurotoxic metabolites of the kynurenine pathway, such as 3-HK and QUIN, are implicated for the responsibility for depressive symptoms (Muller and Schwarz, 2007). In addition, post mortem studies have found microglia activation and decreased number of astrocytes in patients with depression (Si et al., 2004; Steiner et al., 2011). Furthermore, in recent studies, low CSF levels of KYNA associated to severe depressive symptoms (Bay-Richter et al., 2015) and low plasma KYNA has also been found in patients with depressive disorders (Myint et al., 2007; Schwieler et al., 2016). A reduction in the KYNA/QUIN ratio has also been found in both depressed patients and during the remitted phase of MDD (Savitz et al., 2015b). Interestingly, the KYNA/QUIN ratio has been found to positively correlate with larger hippocampal and amygdala volumes in patients with MDD (Savitz et al., 2015a).

1.4 Virus infections and CNS immune responses

Virus infections usually start in the periphery since viruses, including neurotropic viruses, hardly have access to the CNS directly. Viruses may enter the brain only in rare events, such as in cases of disrupted BBB or by release from circulating immune cells, having penetrated the BBB. Following virus invasions, viral molecules are recognized and subsequently trigger a cell-autonomous immune response, which increases the production of various anti-viral proteins and cytokines (Koyuncu et al., 2013).
Previously, the CNS was believed to be an immune privileged site within the entire body (Bailey et al., 2006; Galea et al., 2007), meaning that the CNS is segregated from the peripheral immune system by the BBB. However, recent studies have dramatically modified this idea and revealed a concept of a bidirectional communication between the brain and peripheral immune systems (Trakhtenberg and Goldberg, 2011; Wilson et al., 2010). Interestingly, a new discovery suggests a lymphatic system directly linking the peripheral immune system to the brain (Louveau et al., 2015). Cytokines are able to cross the BBB and serve as communication molecules between the two systems. The innate immune response in the CNS is initiated by microglia, astrocytes and neurons (Carson, 2002; Reiss et al., 2002), which is the first step of the host defense to viruses or infectious agents in the brain. Microglia are widespread in the brain and serve as monitors to identify foreign pathogens via Toll-like receptors (TLR) during CNS immunological process (Olson and Miller, 2004). Astrocytes also express TLRs and release cytokines, chemokines and neurotrophic factors following pathogen recognition (Falsig et al., 2008; Farina et al., 2007). These mediators trigger adjacent cells and further amplify the innate immune response in the CNS. They also alter BBB permeability, hereby allowing immune cells in the blood circulation to easier cross the BBB and participate in the adaptive immune response.

1.5 Immunological aspects

1.5.1 Immunological aspects in schizophrenia

The concept of immune activation involvement in psychotic disorders emerged in early 1900s by Julius Wagner-Jauregg who won the Nobel Prize for his discovery of the importance of therapeutic fever in mental patients (Tsay, 2013). During the last couple of years, this idea has gained increased support and recognition from numerous observations. Several risk factors, such as late winter birth, maternal infections and early childhood infections are all associated with immunological aspects and the development of schizophrenia (Brown and Derkits, 2010; Dalman et al., 2008; Davies et al., 2003). Based on genome-wide association studies (GWAS), a recent meta-analysis study revealed that immune related genes are the most significantly associated with schizophrenia (Aberg et al., 2013). Moreover, a Positron Emission Tomography (PET) study has revealed activation of microglia in schizophrenia (van Berckel et al., 2008). Furthermore, elevated levels of CSF cytokines including IL-1β (Soderlund et al., 2009) and IL-6 (Sasayama et al., 2013; Schwieler et al., 2015) have been observed in patients with schizophrenia. In analogy, postmortem studies have investigated
mRNA and protein levels of cytokines in the frontal cortex in human. Patients with schizophrenia exhibited higher expression levels of IL-1β and TNF-α in Brodmann area 10 (Rao et al., 2013). Similar results have been found in another postmortem study, which discovered upregulated mRNA levels of IL-1β, IL-6 and IL-8 in dorsolateral prefrontal cortex in patients with schizophrenia (Fillman et al., 2013).

1.5.2 Immunological aspects in depression

Depression often occurs together with other diseases, such as atherosclerosis, rheumatoid arthritis and congestive heart failure, all involving chronic inflammatory factors (Evans et al., 2005). Cytokine immunotherapy is a useful treatment strategy for some specific cancer and viral diseases (Ardolino et al., 2015; Mattiello et al., 2015). Patients with cancer or hepatitis C receiving cytokine immunotherapy frequently generate depressive symptoms (Capuron and Dantzer, 2003; Capuron et al., 2002). Moreover, elevated levels of pro-inflammatory cytokines, such as TNF-α, IL-1β and IL-6 have been found in peripheral blood in patients with depression (Miller et al., 2009; Zorrilla et al., 2001). Furthermore, induction of the immune system by administration of cytokines or cytokine inducers leads to depressive symptoms (Dantzer et al., 2008; Reichenberg et al., 2001) and anti-inflammatory therapy may ameliorate the symptoms of depression (Tyring et al., 2006).

1.6 Animal models

Animal models of mental disorders serve as valuable tools in preclinical research, supporting the investigation of underlying neurobiological mechanisms of the disorder.

The criterion of animal models validation is generally categorized as construct, predictive and face validity. Construct validity emphasizes if the animal model provides a rational theoretical construct. Predictive validity refers to the potency of the animal model to predict the efficacy of pharmacological treatments. Face validity corresponds to the accuracy of the animal model to mimic the core symptoms of the disorder in a human condition.

1.6.1 Animal models of schizophrenia

The existing animal models of schizophrenia are not able to predict the whole spectrum of symptoms of schizophrenia. The design of these animal models are based on specific mechanistic and causative hypotheses that related to schizophrenia. A variety of animal behavioral tests, correlating to human conditions, have been established for validating animal
performance. Animal models of schizophrenia can be categorized into four groups: developmental, genetic, pharmacological and lesion models.

PPI of the startle reflex measures the sensorimotor gating and serves as an index in schizophrenia. It measures the ability of a non-startling auditory stimulus to inhibit the startle response to an intense auditory stimulus (Hoffman and Ison, 1980), i.e. the ability of the brain to filter out redundant information (Braff et al., 1978; Braff and Geyer, 1990). It is well known that patients with schizophrenia display disruptions in PPI (Braff et al., 1978; Swerdlow et al., 2008). PPI is a cross-species operation of measuring sensorimotor gating, it has thus been widely used in translational animal models (Swerdlow et al., 2000; Swerdlow et al., 1994).

1.6.1.1 Developmental models

Developmental models of schizophrenia are based on the fact that exposure of the neonate to environmental risk factors during prenatal or postnatal period increases the risk of schizophrenia development. Risk factors, such as infections or immune activation, maternal stress, and isolation rearing perturbed the neonate’s CNS development (Asp et al., 2009; Bitanihirwe et al., 2010; Harms et al., 2008; Holloway et al., 2013; Koike et al., 2009; Macedo et al., 2012).

1.6.1.2 Genetic models

Genetic animal models are instructive for the understanding of the pathogenesis of the disorder. Creating animal models with targeted deletions of the genes of interest is a common strategy to explore the function of a single gene in the pathophysiology of a disorder. One of the historical candidate genes of schizophrenia is disrupted-in-schizophrenia 1 (DISC1). DISC1 knockout (KO) mice display impairments in a broad spectrum of behaviors associated to psychiatric disorders including PPI (Lipina and Roder, 2014). As previously described, KYNA plays an essential role in schizophrenia pathophysiology. Thus, the main biosynthesis enzyme of KYNA (KAT II) has been investigated and KAT II KO mice were developed to study the phenotypic function of low KYNA levels. Indeed, KAT II KO mice display improved cognitive functions (Potter et al., 2010). Another genetic model associated with the kynurenine pathway is the KMO KO mouse model, which presents high levels of brain KYNA (Giorgini et al., 2013). In the present thesis, Kmo−/− mice have served as a platform to study subsequent influences of increased brain KYNA levels.
1.6.1.3 Pharmacological models

Pharmacological models are mainly designed from our current knowledge regarding neurotransmitter changes in schizophrenia. The most well known pharmacological model of schizophrenia is the amphetamine model, which reflect the mesolimbic dopaminergic hyperactivity (Breier et al., 1997; Laruelle, 1998). Administration of amphetamine induces hyperlocomotion and stereotypy in animals (Kokkinidis and Anisman, 1980; Sharp et al., 1987), which have been correlated with positive symptoms of schizophrenia. Moreover, NMDA receptor antagonist such as PCP and ketamine induce not only positive systems, but also partial negative and cognitive deficits of schizophrenia (Marcotte et al., 2001; Monte et al., 2013).

1.6.1.4 Lesion models

Lesion models are generated from the theory that neurodevelopment and neurodegeneration are linked to schizophrenia. Specific brain regions including the prefrontal cortex, the hippocampal formation and the thalamus have been selected when constructing lesion models of schizophrenia (Bardgett et al., 1997; Jaskiw et al., 1990; Kodsi and Swerdlow, 1997; Mittleman et al., 1993; Swerdlow et al., 1995). Lesion models typically involve excitotoxic substances, which lead to the release of excitatory glutamate and cause damage to neuronal tissue. The most acknowledged lesion model is the neonatal lesion model, which is also categorized into developmental models. It is widely used to study the neurodevelopmental theory of schizophrenia (Brake et al., 2000).

1.6.2 Animal models of depression

1.6.2.1 Genetic models

The monoamine hypothesis of depression states a depletion of monoamine neurotransmitters, such as serotonin, norepinephrine, and/or dopamine in the CNS leads to depression (Delgado, 2000). Based on this theory, traditional genetic models of depression such as serotonin receptor 1A knockout mouse model (Heisler et al., 1998; Ramboz et al., 1998) or the noradrenaline transporter knockout mouse model (Xu et al., 2000) were generated. Other transgenic animal models, targeting on corticotropin-releasing hormone receptor-1 (Muller et al., 2003) or the type II glucocorticoid receptor (Montkowski et al., 1995), are based on a theory suggesting a dysregulation of the hypothalamic-pituitary-adrenal (HPA) axis in depression.
Selective breeding is another method to develop animal models of depression. It consists of selection of animals according to their specific features and several generations of breeding. Because of these reasons, animals are genotypically identical to each other in inbred strains and show specific behavioral or physiological abnormalities. One example of genetic models by selective breeding is the Flinders Sensitive Line (FSL) and the Flinders Resistant Line (FRL) rats, which were originally generated towards increased sensitivity to an anticholinesterase agent (diisopropyl fluorophosphates, DFP). Compared to the FRL rats, the FSL rats exhibit partial depressive-like behavior as well as certain neurochemical and pharmacological features involved in depression (Overstreet et al., 2005). The FSL rats display reduced appetite, psychomotor retardation as well as deficits in sleep and immune system (Overstreet et al., 2005; Overstreet and Wegener, 2013).

1.6.2.2 Stress Models
Stress models of depression are based on different types of stress including social stress, chronic mild stress, early life stress and learned helplessness. These different types of stress models offer distinctive information for various subsets of the disorder (Czeh et al., 2016).

1.6.2.3 Immune models
The hypothesis of immunological abnormalities in depression has gained increased attentions. Activation of the peripheral immune system in humans and rodents induces sickness behavior including fever, fatigue, anhedonia, nausea and malaise (McCusker and Kelley, 2013), which have phenomenological similarities with depressive symptoms. LPS, a main component of the out membrane of gram-negative bacteria, has been found to induce sickness behavior in rodents (Bluthe et al., 1994). Administration of LPS induces the expression of pro-inflammatory cytokines in the brain, subsequently activates IDO1 and disrupts the balance of the kynurenine pathway (Parrott et al., 2016). Thus, the LPS model has been used as a reliable immune animal model to study the pathophysiological role of immune activation in depression (O'Connor et al., 2009c; Salazar et al., 2012).
2 AIMS OF THE THESIS

1) To investigate locomotor response to a d-amphetamine challenge in adult wild-type mice neonatally infected with influenza A virus.

2) To investigate whether neonatally elevated brain KYNA is associated with the disturbed behavior in adulthood seen after infection in early life.

3) To investigate if the concentrations of kynurenine pathway metabolites are changed in different brain regions in Flinders Sensitive Line rats, an animal model of depression.

4) To investigate possible changes in gene expression in the brain of mice with a targeted deletion of Kmo ($Kmo^{-/-}$ mice).

5) To assess cerebral and cerebellar variations in KYNA levels in $Kmo$ deficient mice.

6) To characterize $Kmo$ deficient mice behaviorally.

7) To investigate if KAT II KO mice, which have less capacity to generate KYNA, are prevented from elevation of brain KYNA induced by an LPS triggered immune response.
3 MATERIALS AND METHODS

3.1 Animals

In paper I, wild-type C57BL/6 mice (Scanbur AB, Sweden) were used in all experiments. The virus-infected mice and their counterparts were bred at the Department of Neuroscience, Karolinska Institutet, Sweden. The kynurenine-treated mice and their respective controls were bred at the Department of Physiology and Pharmacology, Karolinska Institutet, Sweden. Female FSL and FRL rats were used in paper II and bred at the Department of Clinical Neuroscience, Karolinska Institutet, Sweden. In paper III, male Kmo<sup>−/−</sup> mice and their control wild-type mice were bred on C57BL/6 background at the Department of Genetics, University of Leicester, UK or FVB/N background at the Department of Physiology and Pharmacology, Karolinska Institutet, Sweden as well as the Department of Psychiatry, University of Maryland School of Medicine, Baltimore, USA. The whole genome gene-expression analysis was carried out on Kmo<sup>−/−</sup> and wide-type mice on a C57BL/6 background. Metabolite analyses, enzyme activity and behavior tests were performed on Kmo<sup>−/−</sup> and wild-type mice on a FVB/N background. In paper IV, male KAT II knockout and wild-type mice on a FVB/N background were used in all experiments and bred at the Department of Physiology and Pharmacology, Karolinska Institutet, Sweden.

All animals were maintained under standard laboratory conditions with free access to food and tap water in a light controlled room (12 h light/dark cycle, light on at 6.00 a.m.). Experiments were approved by and performed in accordance with the guidelines of the Ethical Committee of Northern Stockholm, Sweden. In paper III, all experiments were approved and issued by the Home Office (UK), the Institutional Animal Care and Use Committee of the University of Maryland School of Medicine (USA), or 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

L-kynurenine sulfate salt (Sigma Aldrich) was dissolved in sterile dH<sub>2</sub>O and adjusted pH with NaOH to approximate 8.2 (paper I and IV). d-amphetamine (Sigma Aldrich) was dissolved in sterile dH<sub>2</sub>O (paper I and III). LPS (Escherichia coli serotype O111:B4) was purchased from Sigma Aldrich and dissolved in sterile saline (paper IV).
3.3 Treatment

3.3.1 Influenza A/WSN/33 virus infection (paper I)

The mouse adapted neurotropic influenza A/WSN/33 virus was obtained from Dr. S Nakajima (The Institute of Public Health, Tokyo, Japan). Mice were infected intraperitoneally (i.p.) at postnatal day (PND) 3 or PND 4 with 2400 plaque-forming units of the influenza A/WSN/33 virus suspended in 30 µl of phosphate buffered saline (PBS; Gibco) to mimic a hematogenous route of infection.

3.3.2 L-kynurenine treatment (paper I and IV)

In paper I, L-kynurenine was injected i.p. to C57BL/6 mice from PND 7 to PND 16, every 12th hour to mimic a transient increase in brain KYNA levels following influenza virus infection (Holtze et al., 2008). In paper IV, acute L-kynurenine injection was performed i.p. in KAT II KO and wild-type mice.

3.3.3 LPS injection (paper IV)

Both KAT II KO and wild-type mice received two LPS injections (i.p.) at PND 21. The second injection was administered 16 hours after the first injection and mice were sacrificed 24 hours after the first injection at PND 22. In another set of animals, LPS was injected twice in both wild-type and KAT II KO mice as adults.

3.4 Behavior tests (paper I and III)

Several behavior tests were utilized to investigate behavioral alterations in different animal models. Locomotor activity, elevated plus maze, light-dark box and PPI were investigated in both paper I and III. Trace fear conditioning was used in paper I. Passive avoidance and social interaction were investigated in paper III.

3.4.1 Locomotor activity

Locomotor activity was studied in an open field apparatus for mice. Each mouse was placed in a square Plexiglas box (50 x 50 x 21.6 cm) within a sound-dampened and solid chamber. To detect the movement, the chamber was equipped with two rows of photocells sensitive to infrared light forming a two-layer grid over the open field arena. All mice were habituated
during three sessions of 60 min, 24 h apart. General locomotor activity was examined during the first session of the first day. The last habituation session was immediately followed by a test session of 90 min. d-amphetamine or vehicle was injected i.p. approximately 1 min prior to the test session. All habituation sessions and test sessions were performed in the dark during the animal’s light cycle.

3.4.2 Elevated plus maze

The protocol of elevated plus maze was based on the method of Lister (Lister, 1987). The maze was shaped like a “plus” sign and consisted of two open arms (30 × 5 cm) and two equal-sized closed (30 × 5 × 15 cm) arms opposite to each other. Open and closed arms extended from a central platform (5 × 5 cm). The maze was made of stainless steel and mounted on a base, raising it to a height of 50 cm above the floor. Mice were individually placed on the central platform facing an open arm, and allowed to freely explore the maze for 5 min. The number of entries into different arms and the time spent in each arm was recorded by a video camera and then analyzed using the top-view based behavior analysis software TopScan Lite (Clever Sys Inc., Reston, VA, USA). The number of entries and percent time spent in different arms was measured as an index of exploratory behavior. After each test, the maze was cleaned with 70% ethanol solution and dried for 10 min.

3.4.3 Light-dark box

The light-dark box was made of Plexiglass (50 x 25 x 25 cm) and was equally divided into an open white compartment and a darkened closed black compartment. The two compartments were separated by a partition with a 10 x 5 cm opening in the center. Each mouse was placed in the center of the light section facing away from the dark chamber and allowed to explore the box freely for 5 min while being recorded with an overhead digital video camera. The box was cleaned with 70% ethanol solution between subjects. Time spent in each compartment and total number of transitions was analyzed.

3.4.4 Trace fear conditioning

The trace fear conditioning test was carried out by means of a fear conditioning chamber (Med Associates Inc., St. Albans, VT, USA). During training section, mice were allowed to explore the chamber for 100 s before a 20 s tone cue (90 dB). After 18 s interval a foot shock (2 s duration, 0.5 mA intensity) was delivered through the stainless steel rods on the floor of the apparatus. A second tone-shock pairing was repeated after 100 s inter trial interval and the
following 5 s after the second shock was defined as working memory. Working memory is generally defined as cognitive entities (or “central executive” mechanisms) relating to temporary storage and operation of information in both humans and animals (Yoon et al., 2008). The mouse was removed 30 s after the last shock. Three days later, freezing was recorded in the same context, with no tone or shock exposures. Freezing was scored automatically by the Med Associate software and defined as the absence of movement except that required for respiration. Approximately 3h after the contextual assessment, mice were again placed in the apparatus and freezing was recorded in a novel environment (a plastic floor covered the metal grid and a pyramidal shape was inserted in the rectangular box) and in response to the cue (tone). After 100 s exploration, the auditory cue was presented for 20 s followed by a 120 s inter trial interval, then another 20s cue presented. The percentage of time spent freezing was used to score learning and memory. A decrease in percent freezing represents impairment of these abilities.

3.4.5 Passive avoidance

The passive avoidance paradigm (PAP) had two compartments of equal size (each 22 cm high, 18 cm wide and 16 cm deep), one illuminated and the other in darkness, separated by a guillotine door. During the acquisition trial, on day 1, the mouse was first placed in the illuminated compartment. The door was then opened, prompting the mouse to move rapidly into the preferred dark compartment. The latency to enter the dark compartment was recorded as the “approach latency” and the guillotine door was immediately closed. An inescapable foot shock (0.56 mA for 1 sec) was delivered through metal rods of the floor. Twenty-four hours later, in the retention trial, the mouse was again placed in the light compartment, and the guillotine door was opened. The time from opening the guillotine door to the time of entering the dark compartment, which defined as “avoidance latency”, was recorded.

3.4.6 Social interaction

The social interaction test was performed in a three-chambered box. All chambers were equal in size (each 17 cm wide and 29.5 cm deep). The compartments were separated by clear walls with openings to allow access into each chamber. The test mouse was placed into the center chamber and allowed to explore for 5 min, the two side chambers were obstructed by plastic boxes. After acclimatizing to the testing environment, mouse was given 10 min to explore all three chambers of the testing apparatus, this was termed the habituation phase and the amount of time spent in each chamber was recorded. After the habituation period, an unfamiliar
129Sv/Ev male (stranger) was placed in one of the side chambers. The location of the stranger mouse systematically alternated between testing sessions. The stranger mouse was enclosed in a wire cup (9.5 cm in diameter), which allowed nose touching but no fighting. In the alternate chamber, an empty wire cup (termed the novel object) was placed. The test mouse was allowed to explore the entire testing apparatus for a 10 min session. The amount of time spent in each chamber was recorded by two blinded human observers seated five feet away. Data between these two observers were averaged. Additionally, the testing was video recorded, and a third blinded human observer scored the direct interaction between the test mouse and stranger mouse.

3.4.7 Prepulse inhibition

PPI of the startle response is used as an operational measure of sensorimotor gating and is analyzed by measuring the ability of a non-startling “prepulse” to inhibit the response to a startling stimulus (Hoffman and Ison, 1980). Startle response and PPI testing were performed in commercial startle chambers (35 x 33 x 46 cm, SR-LAB™ system, San Diego Instruments, San Diego, CA). The mouse was placed into a Plexiglas cylinder (3.7 cm in diameter), which was mounted in each chamber. Sudden movements by the mouse were detected by a piezoelectric accelerometer attached below the cylinder. A standard personal computer recorded the signals and controlled the presentations of acoustic stimuli and broadband background noise.

The experimental session consisted of a 5 min habituation period to a 65 dB background noise (continuous throughout all blocks), followed by five 120 dB single trials. This was immediately followed by three different test blocks: 1) A variable stimulus intensity block including five trial types: a 40 msec 80, 90, 100, 110, or 120 dB startle pulse, each trial presented 4 times. 2) A variable prepulse intensity block including 12 single 120 dB trials, and 10 trials each of a 69, 72, or 81 dB, 20 msec long prepulse followed 80 msec later by a 120 dB pulse. 3) A variable interstimulus interval block consisting of a 72 dB prepulse followed by a 120 dB pulse with a 25, 50, 100, 200 or 500 msec delay from the start of the prepulse. Eight single 120 dB trials were interspersed within the block.
3.5 Microdialysis (paper I)

3.5.1 Microdialysis surgery

Mice were anesthetized in a Plexiglas chamber continuously ventilated with 4.8% isoflurane in air using a vaporizer (Univentor 400 Anesthesia Unit; Univentor Ltd, Zejtun, Malta) and then mounted onto the ear bars of a conventional stereotaxic frame (David Kopf Instruments, Tujunga, CA, USA). Anesthesia was maintained using a nose cone delivering 2.4% isoflurane. Body temperature was maintained at 37 °C throughout the surgery, by a thermometer and a heating pad (Homeothermic Blanket Control Unit 50–7053-F, Harvard Apparatus, Holliston, MA, USA). An ocular lubricant was applied and 0.5 ml sterile saline was given s.c. to prevent dehydration. The skull was exposed and cleaned from adhesions. Next, a thin layer of quick-setting cyanoacrylate glue (BT AB, Stockholm, Sweden) was applied to the exposed skull, serving as an adhesion surface to the dental cement. A small hole was then drilled over striatum (AP: 0.5 mm anterior to bregma, L: 2 mm from midline), and, following careful removal of the dura, a guide cannula (AT4.7.IC, AgnTho’s AB) containing a dummy probe (outer diameter: 0.2 mm) was implanted (V: 1.5 mm below the brain surface), and secured to the skull with acrylic dental cement (Dentalon® plus, Heraeus, Hanau, Germany). Before each guide cannula implantation the incisor bar was adjusted so that the skull was set in a horizontal flat plane. The wound was then sutured and mice were allowed to recover single-housed for 48 h with food and water ad libitum.

3.5.2 In vivo microdialysis

On the day of experiment, microdialysis was performed in the home cage in unanaesthetized freely moving mice. Mice were tethered to a swivel, the guide was removed and a microdialysis probe (AT4.7.2.PES, shaft length: 7 mm, membrane length: 2 mm, molecular cut-off: 6 kDa, AgnTho’s AB) was inserted through the guide cannula. Probes were perfused with perfusion fluid (Ringer solution containing 148 mM NaCl, 4 mM KCl, 0.8 mM MgCl₂, 1.4 mM CaCl₂), delivered via polyethylene tubing from a microinfusion pump (Univentor 864, Univentor Ltd) at a flow rate of 1 µl/min. All samples were collected in plastic tubes attached to the tether in 30 min (for the analysis of dopamine) intervals throughout the experiment and immediately manually injected (Rheodyne, Cotati, CA, USA) into a high performance liquid chromatography (HPLC) system. To minimize the inter-individual variation due to differences in probe recovery, the dialysate concentrations were transformed to percent of baseline before statistical analysis. A stable baseline, consisting of three
consecutive samples with a maximal variation of 10%, was usually obtained after 2–3 h and defined as 100%. These three samples were taken for determination of basal extracellular dopamine concentrations. Results for subsequent samples were calculated as percentages of this average basal release. The mice then received an i.p. injection of d-amphetamine (2 mg/kg), and dopamine concentrations were measured for up to 300 min. After the session, the mice were sedated with isoflurane and sacrificed with cervical dislocation and tissues were frozen for later analysis and histological verification of probe placement.

3.6 High performance liquid chromatography (paper I - IV)

3.6.1 Fluorescent detection (paper I – IV)

The HPLC system consist of a dual-piston, high-pressure liquid delivery pump Shimadzu LC-10AD (Shimadzu Corporation, Kyoto, Japan), a ReproSil-Pur C18 column (4 × 100 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. Brain samples (50 µl) were manually injected by a Rheodyne® 7725i injector (IDEX, Oak Harbor, WA, USA) into a 100 µl loop. A second mobile phase containing Zinc acetate (0.5 M, not pH adjusted) was delivered after the column at a flow rate of 10 ml/hour by a peristaltic pump (P-500; Pharmacia, Uppsala, Sweden). Signals from the fluorescence detector were transferred to a computer for analysis using Datalys Azur software (Grenoble, France). The retention time of KYNA was about 7–8 min. The sensitivity of the system was verified throughout the session by analysis of KYNA standards.

3.6.2 Electrochemical detection (paper I and II)

The HPLC system is coupled to an electrochemical detector (Coulochem III; ESA Inc., Chelmsford, MA, USA). A mobile phase consisting of 20mM sodium phosphate, 0.7 mM octanesulfonic acid and 10% acetonitrile (pH set to 3.2 using acetic acid) was pumped through a ReproSil-Pur C18 column (4 × 150 mm, Dr. Maisch GmbH), at a flow rate of 0.6ml/min, delivered by a LC-20AD VP HPLC pump (Shimadzu Corporation, Kyoto, Japan). Samples of 20 µl (kept at –25°C until analysis) were manually injected through a Rheodyne® 7725i injector (IDEX) into a 100µl loop. The retention time of 3-HK was about 8.5 min. Signals from the detector were transferred to a computer for analysis with Clarity (DataApex
3.7 Microarray analysis (paper III)

Animals were killed by cervical dislocation, and their brains were rapidly removed and placed on ice. The cerebellum was then separated from the cerebrum, and all samples were snap-frozen in liquid nitrogen and stored at -80°C until use. Tissues were homogenized in TRIzol reagent (Invitrogen, Fisher Scientific, UK Ltd, Loughborough, UK), and RNA was extracted following the manufacturer's protocol. Integrity of total RNA samples was assessed on an Agilent 2100 Bioanalyzer. All samples had an RNA Integrity Number (RIN) >7.5. 250 ng of total RNA were reverse-transcribed to cDNA from which biotin-labelled cRNA was synthesized and purified using an Illumina TotalPrep RNA Amplification kit (Ambion, Fisher Scientific) according to the manufacturer’s instructions. The cRNA was hybridized to MouseWG-6 v2.0 Expression BeadChips, which allow the simultaneous quantification of 45,200 transcripts. The beadchips were scanned on an Illumina BeadArray Reader, and the raw microarray data were extracted using Illumina Genome Studio V2011.1 software. The data were normalized and background subtracted using ArrayTrack.1 Transcripts with low mean expression (fluorescent intensity <150 units, which was equivalent to the mean background fluorescent intensity across the whole slide) across all samples were removed. Data sets were compared by Welch t-test using Arraytrack, and only differentially expressed transcripts with p < 0.05 and >1.2-fold changes were included for subsequent analyses. The differentially-expressed transcript list was annotated with Arraytrack. In order to visualize groups of similarly differentially-expressed transcripts, hierarchically clustered heat maps were produced using GENE-E software.

3.8 Statistics

Data analyses were formed with the statistical software package GraphPad Prism® 6 (GraphPad Software Inc., San Diego, CA, USA) for Mac OS X. A p-value <0.05 was considered statistically significant.

Behavior tests including locomotor activity, trace fear conditioning, passive avoidance and social interaction were analyzed with two-way repeated measure (RM) analysis of variance (ANOVA) followed by Bonferroni post-hoc test. Elevated plus maze and light-dark box tests were analyzed by t-test or Mann–Whitney U test. Biochemical data were analyzed with Mann–Whitney U test in paper I and II, and one-way or two-way ANOVA followed by
Bonferroni post-hoc correction in paper III and IV. Microdialysis data were analyzed with two-way RM ANOVA followed by Bonferroni multiple comparison test. Percent PPI was calculated as follows: ((A-B)/A)*100, where A is the average startle magnitude of the 120 dB trials within each block, and B is the average startle magnitude of the prepulse trial in the same block. Basal average startle magnitude between groups was evaluated with a two-tailed unpaired $t$-test. Differences in percent PPI were calculated with two-way RM ANOVA (genotype x prepulse level or interstimulus interval) followed by Bonferroni post-hoc test.
4 RESULTS AND DISCUSSION

4.1 Paper I

Exposure to infection in early life increases the risk for schizophrenia development. Previous studies reported transient increases of brain KYNA concentration in neonatal influenza A/WSN/33 virus infected wild-type mice (Holtze et al., 2008) as well as in immunodeficient mice (Asp et al., 2010). While the neonatal infection was related to anxiety and impairments in working memory and PPI in adult immunodeficient mice (Asp et al., 2009) no such effects were observed in the wild-type mice (Asp et al., 2010). The aim of this paper is therefore to investigate locomotor response to a d-amphetamine challenge in adult wild-type mice neonatally infected with influenza A virus as well as to investigate whether neonatally elevated brain KYNA is associated with long-term behavior disturbances seen after infection in early life.

4.1.1 Neonatal virus infection or neonatal L-kynurenine administration enhance locomotor responsiveness to d-amphetamine in adult mice

The present study demonstrates that acute administration of d-amphetamine (5 mg/kg, i.p.) in adult life increased horizontal activity in neonatally influenza A/WSN/33 virus-infected mice and their respective controls. As compared to the uninfected control mice, neonatal infected mice displayed a more pronounced increase in d-amphetamine-induced locomotor response (Figure 2). The basal horizontal activity did not differ between neonatally virus-infected mice and their uninfected controls. Similar results were obtained in adult mice neonatally injected with kynurenine (Figure 3).
Figure 2. Horizontal activity during habituation three and following acutely administered d-amphetamine (5 mg/kg) or vehicle to 5–6 months old mice, injected with influenza A virus (2400 plaque-forming units) or phosphate buffered saline (PBS) at PND 3 or 4. Each point represents the mean ± S.E.M. of counts recorded during five-minute intervals. Statistical analysis was performed by a two-way ANOVA for repeated measurements (time × treatment) followed by Bonferroni’s multiple comparison test (Interaction: F (51, 493) = 3.629, p < 0.001; Time: F (17, 493) = 25.34, p < 0.001; Treatment: F (3, 29) = 16.29, p < 0.001).

Figure 3. Horizontal activity during habituation three and following acutely administered d-amphetamine (5 mg/kg) or vehicle to 3–4 months old mice, injected with L-kynurenine (2 × 200 mg/kg/day) or saline from PND 7 to 16. Each point represents the mean ± S.E.M. of counts recorded during five-minute intervals. Statistical analysis was performed by a two-way ANOVA for repeated measurements (time × treatment) followed by Bonferroni’s multiple comparison test (Interaction: F (51, 204) = 2.631, p < 0.001; Time: F (17, 204) = 10.61, p < 0.001; Treatment: F (3, 12) = 8.788, p < 0.01).
4.1.2 Neonatal kynurenine treatment disrupts prepulse inhibition and working memory in adult mice

Neonatally L-kynurenine treated mice displayed mild reductions in PPI as adults (Figure 4). Significant disruption in PPI was only seen at the 500 ms interstimulus interval, suggesting reduced processing time in mice neonatally exposed to L-kynurenine. Furthermore, neonatally L-kynurenine treated mice showed a reduction in working memory in trace fear conditioning test as adult (Figure 5). However, no significant difference in contextual memory was found, implying a partial disruption of the hippocampus and/or other related brain regions in this developmental animal model. In line with the paper by Chess and colleagues (Chess et al., 2009), no tone-cued memory alterations were observed.

Figure 4. Prepulse inhibition (PPI) during the varied interstimulus interval (ISI) block of the startle session in 3–4 months old mice, neonatally injected with L-kynurenine (2 × 200 mg/kg/day, n = 8) or saline at PND 7–16 (n = 10). Data are presented as mean ± S.E.M. **p < 0.01.

Figure 5. Trace fear conditioning of L-kynurenine treated and saline treated C57BL/6 female mice. Percent component time freezing during the training trial was analyzed in the following time periods: Baseline, first tone cue (Tone1), Inter-trial interval (ITI), second tone cue (Tone2) and working memory (WM). Data were analyzed with two-way ANOVA for repeated measurements (time × pretreatment) followed by Bonferroni multiple comparison test, which revealed a significant impairment of working memory in L-kynurenine treated mice, as compared to saline treated controls, (Treatment: F (1, 22) = 24.82, ***p < 0.001).
4.2 Decreased levels of prefrontal cortex KYNA in a genetic animal model of depression (Paper II)

In this paper, two key metabolites of the kynurenine pathway were analyzed in different brain regions of a rat model of depression, i.e. the FSL and FRL. Varied KYNA levels were detected in the brain areas of both FSL and FRL rats. No changes in KYNA levels in the frontal cortex, striatum, hippocampus or cerebellum were observed between the two strains. However, in the prefrontal cortex the concentration of KYNA levels was significantly lower in the FSL compared to FRL rats (Table 1).

Levels of 3-HK were analyzed in the prefrontal cortex and the frontal cortex in these rats and no differences were found between the two strains. As a result, the KYNA/3-HK ratio was significantly lower in the prefrontal cortex in FSL rats compared to FRL rats. These results indicate an imbalanced metabolism between the two main branches of the kynurenine pathway in this animal model of depression. The region specific abnormality of the kynurenine pathway metabolism implies an essential role of the prefrontal cortex in MDD. Indeed, several imaging studies have demonstrated abnormalities in neurophysiology of the prefrontal cortex in patients with MDD (Dutta et al., 2014; Johnstone et al., 2007; Liu et al., 2014b).

| Table 1. Levels of KYNA, 3-HK and KYNA/3-HK ratio in different brain regions of FSL and FRL rats |
|-----------------------------------------------|-----------------|-----------------|-----------------|-----------------|
| KYNA                                         | 3-HK            | KYNA/3-HK ratio |
| FSL (n=10)                                   | FRL (n=10)      | FSL (n=8)       | FRL (n=8)       | FSL (n=10)      | FRL (n=10)      |
| Frontal Cortex                               | 10.0 [4.3-15.6] | 8.5 [6.5-27.8]  | 21.6 [16.3-27.8]| 19.9 [7.4-31.5]| 0.39 [0.2-0.6]  | 0.66 [0.2-3.6]  |
| Prefrontal cortex                            | 3.8 [3.1-5.9]   | 14.5 [5.1-32.1] | 14.2 [12.5-19.1]| 12.8 [9.1-17.8]| 0.25 [0.2-0.4]| 1.0 [0.4-2.1] |
| Striatum                                     | 3.5 [2.7-4.7]   | 4.0 [3.1-16.6]  | -               | -               | -               | -               |
| Hippocampus                                  | 10.3 [2.5-21.1]| 8.5 [5.5-30.6]  | -               | -               | -               | -               |
| Cerebellum                                   | 4.9 [3.6-6.4]   | 5.3 [3.4-7.6]   | -               | -               | -               | -               |

Median concentrations [IQR] of KYNA and 3-HK are expressed in nM. Numbers of animals are given in brackets. Differences in KYNA, 3-HK levels and KYNA/3-HK ratios between FSL and FRL rats were evaluated by Mann-Whitney U test followed by the Bonferroni test for multiple comparison, *p<0.05, **p<0.01.
4.3 Paper III

In this study, mice with a targeted deletion of KMO were recruited to explore gene expression changes and behavioral alterations compared to wild-type mice.

4.3.1 Differential gene expression profiling identifies a network of schizophrenia-related genes in $Kmo^{-/-}$ mice

In order to explore the regulatory changes in the $Kmo^{-/-}$ mice, an unbiased screen for differentially expressed genes (DEGs) was performed. Gene profiling identified a number of DEGs in both cerebrum and cerebellum ($p \leq 0.05$). Of the two samples, the cerebrum exhibited a greater number of DEGs in $Kmo^{-/-}$ mice than in wild-type mice, with a total of 120 DEGs. In the cerebellum, a set of 24 genes was identified (Figure 6). However, only 6 genes overlapped between the forebrain and the cerebellum: CNIH4, FCER1G, LYPLAL1, MGST3, MYOC and SLC22A6. Interestingly, several identified genes have previously been implicated as dysfunctional in schizophrenia. In the cerebellum, we identified more upregulated genes associated with schizophrenia, but, in the cerebrum, identified DEGs that are linked to schizophrenia were more evenly distributed between up- and down-regulated categories.
Figure 6. Hierarchical clustering (Euclidian distance) was used to visualize the gene-expression changes relative to other genes in the dataset. Expression changes were sorted into clusters of similarly expressed genes both inter- and intra-dataset, across different brain regions in $Kmo^{-/-}$ relative to wild-type mice. Blue indicates a relative decrease whereas red indicates an increase. A greater number of significant gene-expression changes were observed in the cerebrum. Genes with significant ($p<0.05$) fold changes $>1.2$ are included in the analysis ($n=4$ per genotype).
Network analysis revealed a single robust interaction network in several functional clusters (Figure 7), supporting the idea that a large amount of the DEGs arise from genomic abolition of KMO activity – hereby linked to increased KYNA levels – and act in a common network.

**Figure 7.** Differentially expressed genes (DEGs) form a highly interconnected network. Network analysis determined the DEGs identified in *Kmo^-/-* mice form a robust network containing 67/144 of the candidates. The network is characterized by several functional clusters highlighted with different colors.
4.3.2 Brain KYNA levels are elevated in Kmo<sup>−/−</sup> mice

KMO activity, 3-HK levels and KYNA levels were measured in both cerebrum and cerebellum of Kmo<sup>−/−</sup> mice in order to compare kynurenine pathway changes in these two brain areas. KMO activity (Figure 8A) and the amount of its enzymatic product 3-HK (Figure 8B) were dramatically reduced in both cerebrum and cerebellum of Kmo<sup>−/−</sup> mice. Conversely, KYNA levels were significantly elevated in both brain areas in Kmo<sup>−/−</sup> mice, and unexpectedly, we observed higher KYNA levels in the cerebellum than in the cerebrum (Figure 8C).

![Figure 8](image)

**Figure 8.** Kynurenine pathway metabolism in cerebrum and cerebellum of adult wild-type and Kmo<sup>−/−</sup> mice. (A) KMO activity is eliminated in both tissues in Kmo<sup>−/−</sup> mice. (B) Levels of 3-HK are reduced in both tissues in Kmo<sup>−/−</sup> compared to wild-type mice. (C) Levels of KYNA are elevated in Kmo<sup>−/−</sup> mice. KYNA levels are significantly more elevated in the cerebellum than in the cerebrum. All data are the mean ± SEM. **p<0.01; ***p<0.001; n=5-8 per group.

4.3.3 Kmo<sup>−/−</sup> mice display deficits in contextual memory and social interaction

Contextual memory was assessed in the passive avoidance task, a hippocampus-mediated behavioral test. Kmo<sup>−/−</sup> mice didn’t display behavioral difference with wild-type mice in approach latencies during the acquisition trial (Figure 9A). However, after 24 hours, the avoidance latencies of Kmo<sup>−/−</sup> mice were significantly shorter than those of wild-type mice (Figure 9A). Deficits in hippocampus-dependent contextual memory are in line with the demonstration that elevated brain KYNA is associated with abnormalities in hippocampus-dependent learning and memory (Chess et al., 2009; Pocivavsek et al., 2012; Pocivavsek et al., 2011).

Social interaction was characterized with the three-chamber social approach apparatus. Both wild-type and Kmo<sup>−/−</sup> mice preferred to spend more time with the stranger than the novel object. Notably, when compare with wild-type animals, Kmo<sup>−/−</sup> mice spent a lower percentage
of time with the stranger mouse versus the novel object, suggesting a deficit in social interaction (Figure 9B). This observation is in agreement with data showing impaired social behavior in rats after kynurenine injection during early postnatal development or adolescence (Iaccarino et al., 2013; Trecartin and Bucci, 2011).

Figure 9. Contextual memory and social interaction. (A) Wild-type (n=14) and Kmo<sup>−/−</sup> (n=7) mice were tested in the passive avoidance paradigm. No genotypic difference in approach latency was observed on the training day. On Day 2, only wild-type animals showed contextual memory, i.e. a significant difference between avoidance and approach latency. Avoidance latency differed significantly between wild-type and Kmo<sup>−/−</sup> animals. (B) Performance of wild-type (n=12) and Kmo<sup>−/−</sup> (n=12) mice in the three-chambered social interaction paradigm. Compared to wild-type animals, mutant animals spent a lower proportion of time with the stranger mouse than with the novel object. All data are the mean ± SEM. *p<0.05; *** p<0.001.

4.3.4 Kmo<sup>−/−</sup> mice display anxiety-like behaviors

Similar with previous studies demonstrating an increase in anxiety-like phenotypes after acute or repeated systemic kynurenine administration in rodents (Olsson et al., 2012a; Salazar et al., 2012; Vecsei and Beal, 1990), Kmo<sup>−/−</sup> mice also displayed increased anxiety-like behaviors in the elevated plus-maze, the light-dark box, and open field tests. In the elevated plus-maze, Kmo<sup>−/−</sup> mice showed significant reductions in the percentage of time spent in open arms (Figure 10A) and in the number of entries into open arms (Figure 10B) compared to wild-type mice. In the light-dark box test, Kmo<sup>−/−</sup> mice spent significantly less time in the light compartment compared to their wild-type counterparts (Figure 10C) and made a decreased number of entries into the light compartment (Figure 10D). Furthermore, we observed a significant increase in the corner time of the Kmo<sup>−/−</sup> animals in a general assessment of locomotion (Figure 10E). Together, these data demonstrate increased anxiety-like behaviors in Kmo<sup>−/−</sup> mice compared to wild-type mice.
Figure 10. Anxiety behavior in elevated plus maze (A, B), light-dark box (C, D), and open field (E). In the elevated plus maze, *Kmo<sup>−/−</sup> mice (n=12) spent significantly less time in the open arm (A) and entered the open arms less frequently (B) than wild-type animals (n=12); In the light-dark box, *Kmo<sup>−/−</sup> mice (n=17) spent significantly less time in the light compartment (C) and entered the light compartment less frequently (D) than wild-type mice (n=24); (E) In the open field, *Kmo<sup>−/−</sup> mice (n=21) spent more time in the corners than wild-type animals (n=23). All data are the mean ± SEM. *p<0.05, **p<0.01, ***p<0.001, ****p<0.0001.
4.3.5 *Kmo*<sup>−/−</sup> mice show enhanced locomotor response to d-amphetamine

Acute administration of d-amphetamine (5 mg/kg, i.p.) produced increased horizontal activity compared to saline treated mice and potentiated the increase in horizontal activity in *Kmo*<sup>−/−</sup> mice as compared to wild-type mice (Time: F(29, 1160)=15.24, p<0.0001; Genotype: F(3, 40)=35.55, p<0.0001; Interaction: F(87, 1160)=17.88, p<0.0001, **Figure 11A**). Central activity, indicating the movement of the animal in the center of the cage, in *Kmo*<sup>−/−</sup> mice was enhanced by acute administration of d-amphetamine compared to wild-type mice (Time: F(29, 1160)=6.416, p<0.0001; Genotype: F(3, 40)=11.85, P<0.0001; Interaction: F(87, 1160)=6.117, p<0.0001, **Figure 11B**). This enhanced response is also seen in mice with experimentally induced chronic elevations in brain KYNA levels (Olsson et al., 2009).

![Graph A](image1)

**Figure 11.** Increased locomotor activity after d-amphetamine (AMPH; 5 mg/kg). At time 0 (arrows), animals received an i.p. injection of either AMPH (wild-type: n=12; *Kmo*<sup>−/−</sup>: n=11) or saline (wild-type: n=11; *Kmo*<sup>−/−</sup>: n=10). AMPH increased both horizontal (A) and central (B) activity significantly more in *Kmo*<sup>−/−</sup> mice than in wild-type animals. All data are the mean ± SEM. *p<0.05, **p<0.01, ***p<0.001, ****p<0.0001 versus wild-type.
4.4 Paper IV

In the present study, mice with a targeted deletion of KAT II, hereby having less capacity to generate KYNA, were used as an experimental model to investigate if these mice are prevented from elevation of brain KYNA induced by an LPS triggered immune response. It has been shown that KAT II KO mice have lower levels of KYNA than wild-type mice before PND 28 (Yu et al., 2004).

4.4.1 Brain KYNA levels are lower in KAT II KO mice than in wild-type mice at PND 22

Baseline KYNA levels were measured in wild-type and KAT II KO mouse brains at PND 22 as well as in adults. KAT II KO mice exhibited lower brain KYNA levels compared to wild-type mice at PND 22 (mean ±SEM: wild-type: 1.42 ±0.41 nM (n=16); KAT II KO: 0.25 ±0.05 nM (n=16), **p<0.01, Figure 12), but no difference in baseline brain KYNA was found in the adult animals.

![Figure 12](image.png)

**Figure 12.** Baseline brain KYNA levels were measured in 22 days old wild-type (WT) and KAT II KO mice. n=16 in each group. Data are presented as mean ± SEM and analyzed with unpaired t test. **p<0.01

4.4.2 Administration of kynurenine or repeated LPS injections elevate brain KYNA levels in both wild-type and KAT II KO mice at PND 22.

Brain KYNA levels were measured after kynurenine injection (20mg/kg or 40mg/kg) or repeated LPS injections (2 × 0.83mg/kg). Surprisingly, brain KYNA levels were elevated in both wild-type and KAT II KO mice after kynurenine injection (Figure 13A) or repeated LPS injections (Figure 13B). These results imply that other enzymes aside from KAT II might be responsible for the transamination of kynurenine to KYNA. In order to explore the
underlying mechanisms behind this unexpected observation, mRNA expression levels of the four KAT enzymes were measured.

4.4.3 Repeated LPS injections upregulate KAT III mRNA expression in wild-type and KAT II KO mice at PND 22.

By examining the mRNA expression level of KAT I/II/III and IV enzymes, significant effects of genotype were found in KAT I (F (1, 20) = 116.0, p<0.0001, Figure 14A), KAT III (F (1, 20) = 57.07, p<0.0001, Figure 14C) and KAT IV expression levels (F (1, 20) = 9.750, p=0.0054, Figure 14D). As expected, no KAT II mRNA was detected in KAT II KO mice (Figure 14B). Repeated LPS injections did not affect KAT I, KAT II or KAT IV expression levels. Rather, this treatment was associated with increased KAT III mRNA expression (Treatment: F (1, 20) = 34.34, p<0.0001, Figure 14C) in both wild-type and KAT II KO mice at PND 22 (Wild-type LPS+LPS vs Wild-type saline+saline: ***p<0.001; KO LPS+LPS vs KO saline+saline: *p<0.05, Figure 14C).
Further, in order to investigate if LPS induces mRNA expression of KAT III or if increased concentration of kynurenine per se serves as the inducer, we investigated mRNA expression of KAT III following kynurenine administration. mRNA expression levels of all the four KAT enzymes were analyzed following administration of kynurenine and no significant changes were found between mice administered kynurenine and saline-treated mice (Figure 15).

**Figure 14.** Brain mRNA expression levels of KAT I (A), KAT II (B), KAT III (C) and KAT IV (D) enzymes in wild-type (WT) and KAT II KO mice brain following repeated LPS injections (2 × 0.83mg/kg) at adult age. n=5-7 per group. Data are presented as mean ± SEM and analyzed using two-way ANOVA followed by Bonferroni’s multiple comparisons test. *p<0.05, **p<0.01, ***p<0.001, ****p<0.0001.
4.4.4 Repeated LPS injections upregulate KAT III mRNA expression in KAT II KO and wild-type mice at adult age.

mRNA expression levels of KAT I/II/III and IV enzymes were measured in adult wild-type and KAT II KO mice brains. Significant genotype effects were found in KAT I (F (1, 20) = 12.50, p=0.0021, Figure 16A), KAT III (F (1, 20) = 15.00, p=0.0009, Figure 16C) and KAT IV (F (1, 20) = 25.68, p<0.0001, Figure 16D) mRNA expression levels. KAT II mRNA was not detected in KAT II KO mice (Figure 16B). Similar as the result from PND 22, repeated LPS administration induced KAT III expression (Treatment: F (1, 20) = 27.66, p<0.0001, Figure 16C) in both genotypes at this age (Wild-type LPS+LPS vs Wild-type saline+saline: *p<0.05; KO LPS+LPS vs KO saline+saline: **p<0.01, Figure 16C). However, when comparing the baseline levels of the four enzymes between the two genotypes, KAT II KO mice showed higher KAT III (Wild-type saline+saline vs KAT II KO saline+saline: *p<0.05, Figure 16C) and KAT IV (Wild-type saline+saline vs KAT II KO saline+saline: **p<0.05, Figure 16D) mRNA expression levels than their wild-type counterparts. These results are in
line with a previous study, showing an increased KAT III mRNA levels in KAT II KO mice at PND 60 (Yu et al., 2006).

**Figure 16.** Brain mRNA expression levels of KAT I (A), KAT II (B), KAT III (C) and KAT IV (D) enzymes in wild-type (WT) and KAT II KO mice brain following repeated LPS injections (2 × 0.83mg/kg) at adult age. n=5-7 per group. Data are presented as mean ± SEM and analyzed using two-way ANOVA followed by Bonferroni’s multiple comparisons test. *p<0.05, **p<0.01, ***p<0.001.
5 GENERAL DISCUSSION

During the last 15 years, growing evidence of an imbalanced regulation of the kynurenine pathway in the pathophysiology of psychiatric disorders has emerged. Thus, as shown in numerous studies, elevated levels of brain KYNA are found in patients with schizophrenia and bipolar disorder (Erhardt et al., 2001a; Linderholm et al., 2012; Miller et al., 2006; Nilsson et al., 2005; Olsson et al., 2010; Sathyasaikumar et al., 2011; Schwarcz et al., 2001) and shown to specifically correlate with psychotic features (Lavebratt et al., 2014; Olsson et al., 2012b; Sellgren et al., 2015) and impairments in cognitive functions (Sellgren et al., 2015). Reductions in both peripheral and brain KYNA concentrations have been observed in depressed patients (Bay-Richter et al., 2015; Savitz et al., 2015b; Schwarcz et al., 2012). In suicidal attempters, increased QUIN and decreased picolinic acid (PIC) has been observed (Bay-Richter et al., 2015; Brundin et al., 2016; Erhardt et al., 2013). Thus, the balance between the neuroprotective branch (i.e. KYNA) and the neurotoxic branch (i.e. QUIN), in all probability in combination with a genetic vulnerability, seems to be important for the development of either psychosis or depression.

Experimental studies support the theory that KYNA associates to psychosis and cognitive dysfunctions and that QUIN associates to depression. Thus, it has previously been shown in rodent models that increased levels of brain KYNA disrupt PPI (Erhardt et al., 2004), increase midbrain dopaminergic activity (Erhardt and Engberg, 2002; Erhardt et al., 2001b; Linderholm et al., 2007; Nilsson et al., 2006) and enhance amphetamine-induced dopamine release (Olsson et al., 2009) as well as the locomotor activity (Olsson et al., 2012a). Also cognitive dysfunctions such as impairments in contextual processing, spatial working memory and contextual memory have been found to associate with elevated levels of KYNA in several animal studies (Alexander et al., 2012; Chess et al., 2009; Chess et al., 2007; Pocivavsek et al., 2011). A previous study has shown that KAT II KO mice, i.e. mice with reduced capacity to produce KYNA, display improved cognitive function (Potter et al., 2010). With regard to animal models related to depression, it has been shown that IFN-α induces depressive-like behavior through the induction of the kynurenine pathway, and that this effect is accompanied by elevated hippocampal QUIN levels in the rat (Fischer et al., 2015). Also, chronic stress increases hippocampal QUIN levels and induces depressive-like behaviors in rats. Local administration of QUIN in the hippocampus results in similar depressive-like behaviors (Chen et al., 2013). In addition, LPS has been used as a reliable stimulator to construct an animal model of depression. Thus, several studies have shown that
both peripheral and local administration of LPS induces depressive-like behaviors in rodent (Fu et al., 2010; Lawson et al., 2013; O'Connor et al., 2009c; Salazar et al., 2012). In fact, also administration of a low dose of kynurenine has been shown to induce depressive-like behaviors (Agudelo et al., 2014). The same study also found that physical exercise induces antidepressant effect by inducing the expression of KATs, the enzymes responsible for the conversion of kynurenine towards KYNA, in skeletal muscle in both human and mouse (Agudelo et al., 2014).

In the present thesis, several animal models were used to investigate the role of kynurenine pathway in schizophrenia and depression. The results show that Kmo−/− mice, with high brain KYNA levels, display impairments in cognitive functions and social interaction as well as increased anxiety-like behaviors and enhanced locomotor activity following d-amphetamine administration. These data are in line with our findings in mice neonatally infected with influenza A virus or neonatally treated with kynurenine. Thus, neonatal administrations of neurotropic influenza A virus in wild-type mice lead to enhanced locomotor responsiveness to d-amphetamine. We have previously shown that such a neonatal infections causes a transient elevation of brain KYNA concentrations in mice (Asp et al., 2010; Holtze et al., 2008).

Here, we further investigated the association between elevated KYNA levels in early life and disturbed behaviors in adulthood. Thus, kynurenine, the precursor of KYNA was administrated to wild-type mice neonatally. Indeed, brain KYNA elevation in neonatal period was found to induce impairments in PPI, working memory as well as a tendency to induce hyper dopaminergic responsiveness to d-amphetamine.

In summary, findings of the present thesis reveal a critical role of KYNA in the development of schizophrenia. In support, several studies have shown that elevated levels of KYNA contribute to the neurochemical and behavioral abnormalities in neurodevelopmental animal models (Alexander et al., 2013; Liu et al., 2014a; Pershing et al., 2015; Pocivavsek et al., 2012). Taken together, these findings are in line with the neurodevelopmental theory of schizophrenia, suggesting that environmental insults in early life is a risk factor for schizophrenia development and neonatal elevation of brain KYNA levels might serve as a link between early life environmental insults and disturbed behaviors in adulthood.

The underlying reason for the increase in brain KYNA concentration in schizophrenia and bipolar disorder with psychotic features may be related to polymorphisms in the KMO gene (Holtze et al., 2012; Lavebratt et al., 2014; Wonodi et al., 2011). Interestingly, our genome-
wide differential gene expression analyses in \( Kmo^- \) mice identified a network of schizophrenia- and psychosis-related genes.

In addition, enhanced secretion of pro-inflammatory cytokines may also be important for the observed increase in brain KYNA. Thus, elevated levels of IL-1\( \beta \) have been observed in first-episode schizophrenia patients and in patients with bipolar disorder (Beumer et al., 2012; Dowlati et al., 2010; Lindqvist et al., 2009; Schwieler et al., 2015; Sellgren et al., 2015; Soderlund et al., 2011; Soderlund et al., 2009), whereas elevated levels of IL-6 have been shown in chronic schizophrenia, depression and suicidal attempters (Erhardt et al., 2013; Sasayama et al., 2013; Schwieler et al., 2015). Indeed, pro-inflammatory cytokines induce the kynurenine pathway by activating rate limiting enzymes, IDO1 and TDO2 (Carlin et al., 1987; Miller et al., 2006; Sellgren et al., 2015; Yoshida et al., 1986). Cytokines, such as IFN-\( \gamma \), IFN-\( \alpha \), IFN-\( \beta \) and TNF-\( \alpha \), have been shown to induce IDO1 in several studies (Guillemin et al., 2001a; Pemberton et al., 1997). Recently, studies have shown that IL-1\( \beta \) has the potency to induce TDO2 (Sellgren et al., 2015; Urata et al., 2014). In fact, post mortem studies of patients with schizophrenia and patients with bipolar disorder with psychotic features have found increased expression of TDO2. In the present study, LPS was used as a tool to induce the secretion of pro-inflammatory cytokines. We have recently shown that a double-shot of LPS induces not only the kynurenine pathway but also increase brain KYNA levels (Larsson et al., 2016). Here, KAT II KO mice were used to investigate if loss of KAT II could prevent the increase of KYNA induced by a double-shot of LPS. To our big surprise, repeated injections of LPS up-regulated brain KYNA levels in not only wild-type mice but also in KAT II KO mice. Indeed, mRNA expression data show evidence of increased KAT III mRNA levels in both KAT II KO and wild-type mice. These data imply that under pathophysiological conditions, where the central immune system is triggered, KAT III might be the most prominent enzyme converting kynurenine towards KYNA.

The outcome of the present thesis provides novel information regarding underlying pathological mechanisms in schizophrenia and depression. These findings may have the potential to lay grounds for novel pharmacological interventions, targeting on specific kynurenine pathway enzymes.
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