Research objective 2 - Study of naturally occurring modulators of glutamatergic neurotransmission

Activity 1 – Study of Kynurenine Pathway Metabolism: Shared theory of affective and psychotic disorder pathophysiology

The aim is to investigate the role of perinatal and adult immune stimulation in activation of kynurenic pathway (KP) for brain metabolome by biochemical analysis of brain content and behavioral study in the animal models. Cytokines promote protein synthesis of KP enzymes and formation of kynurenine (KYNA) and quinolinic acid (QUIN). We hypothesize that psychiatric disorders namely bipolar disorder and schizophrenia share a common pathophysiology based on immune dysregulation leading to alternation in KP metabolisms.

Specific aims:

- Kynurenine metabolites differentially affect glutamatergic transmission with KYNA inhibiting while QIUN potentiating NMDAR function. This opposing modulation of NMDARs should be detectable in behavioral tests. Therefore we aim to assess how immune system activation affects various domains of behavior known to reflect altered NMDAR function.
- To investigate the role of perinatal and adult immune stimulation in activation of kynurenine pathway by biochemical analysis of brain content. Lipopolysacharid (endotoxin LPS), the most abundant component of Gram-negative bacteria cell walls, stimulates the release of cytokines thus mimicking bacterial infection. Cytokines promote protein synthesis of kynurenine pathway enzymes and formation of KYNA and QUIN.
- To investigate the effect of "second hit" on already developed schizophrenia-like or depression-like phenotype induced by immune stimulation. We believe that prior immune activation will result in increased susceptibility to the subsequent adverse insult ("second hit"). We will evaluate the effect of NMDAR antagonist (MK-801) administration to adult rats perinatally treated with LPS. Similarly we will examine immune changes following bulb removal (olfactory bulbectomy, OBX) and the effect of systemic acute LPS administration to OBX animals. OBX is an animal model of depressive disorder resulting in depression-like phenotype with long-lasting pro-inflammatory effects
- To determine whether the behavioral and biochemical response to LPS, and reactivity to NMDA antagonists has the circadian component and could be affected by chronopharmacological intervention.
- To investigate whether the levels of cytokines, biochemical markers and behavioral changes could be ameliorated by the administration of immunomodulatory melatonin.

The kynurenine pathway has received increasing attention as its connection to inflammation, the immune response, and neurological condition became more apparent. Kynurenine pathway represents an alternative metabolic pathway of tryptophan catabolism (Richard et al., 2009) to otherwise well understood pathway leading to synthesis of serotonin and melatonin. Tryptophan may be metabolized in glial cells by indoleamine 2,3-dioxygenase (IDO) or tryptophan 2,3-dioxygenase (TDO) enzymes to form kynurenine (KYN) (Davis and Liu, 2015). TDO is responsible for the oxidative degradation of tryptophan under normal physiological conditions, whereas IDO is activated by stimulated immune system (Larkin et al., 2016). IDO is not constitutively expressed thus the expression is induced only upon activation of its transcription factors. Signaling pathways upstream of these transcription factors participate in regulation of IDO activity. Stimulation of TLR3 and TLR4 receptors leads to expression of pro-inflammatory cytokines

resulting in transcription and translation of IDO protein (Mbongue et al., 2015). The clinically relevant consequence of its upregulation is the lack of free tryptophan for the synthesis of neurotransmitter serotonin and its metabolite melatonin.

Kynurenic acid is synthesized from KYN by kynurenine aminotransferase (KAT) mainly in astrocytes whereas quinolinic acid (QUIN) synthesis from kynurenine is catalyzed by kynurenine monoxygenase (KMO) in microglia, monocytes or macrophages. These substances are endogenous modulators of NMDA glutamate receptors where KYNA acts as a NMDA receptor antagonist at glycine site and QUIN as NMDA receptor agonist (Davis and Liu, 2015). Astrocytes synthesize kynurenic acid (KYNA) because they lack the enzyme KMO (Guillemin et al., 2001). By acting on glutamatergic neurotransmission, these neuroactive metabolites play a key role in the development of depression-like and schizophrenia-like behaviors. Indeed, KYN and KYNA levels were found to be elevated in CSF, in the prefrontal cortex and anterior cingulate cortex of patients with schizophrenia. Inhibition of NMDA receptors by KYNA may negatively influence glutamatergic transmission leading to manifestation of schizophrenia symptoms. Dysregulation of the KP is documented also in depressive patients however contrary to schizophrenia quinolinic acid (QUIN; synthesized from KYN) levels are increased.

The immune system is activated in both diseases and upregulation of kynurenine pathway enzymes might be thus detected in schizophrenia as well as depression. Divergent effect of immune activation on the kynurenine metabolism depends on type-specific cytokine associated either with schizophrenia or depression. Increased proinflammatory type-1 cytokines were found in major depression while schizophrenia is characterized by type-1/type-2 cytokine imbalance (Schwarz et al., 2001). Th2 dominant immune response (Th2 shift) found in the subpopulation of schizophrenic patients (Chiang et al., 2004; Avguštin et al., 2005) is associated with overexpression of TDO and the activation of astrocytes leading to accumulation of KYNA. On the other hand Th1 dominant immune response is more frequently found in major depression where IDO and KMO are induced by the type-1 cytokines. Similarly; in those schizophrenics with Th1 dominant immune response the kynurenine pathway changes would be more similar to those found in depression (Muller et al., 2009).

Recent preclinical research revealed that high doses of immunomodulatory melatonin are neuroprotective and diminishes the negative effect of QUIN. It is worthwhile to investigate whether melatonin will alleviate the effect of LPS-induced IDO activation in neural development.

Activity 2 – The role of neurosteroid in development of behavioral disease

Endogenous neurosteroids modulate cortical development. Then alterations in neurosteroid levels may contribute to abnormal neurodevelopment. The aim is to investigate the role of neurosteroid after perinatal immune stimulation (application of LPS) and exposure to stress by biochemical analysis of brain content and behavioral study in the neurodevelopmental animal models of schizophrenia and depression. We hypothesize that augmentation of the neuroactive steroids during development can represent novel prophylactic pharmacotherapy of behavioral disease.

Specific aims:

- Maternal exposure to stress during pregnancy (known alterate endogenous neurosteroid levels) results in behavioral deficits in adult offspring such a decreased ultrasonic vocalization (marker of social deficit) and decreased prepulse inhibition.

- Similar alterations in perinatal neurosteroid levels in the brain could occur in response to severe stress.
- Concerning the neuroprotective role of neurosteroid effects in the fetal brain, there might be also correlations between fetal steroid concentration and development of the central nervous system , hypothalamic-pituitary-adrenal (HPA) in the subsequent stages of development. We suppose that neurosteroids are part of neuroprotective mechanisms protect the fetal brain from hypoxia/ischemia and along with promote neurodevelopment. These deficiences in neurosteroid exposure may contribute to the increase in incidence of the adverse patterns of behavior seen in children that are bornpreterm
- Endogenous neurosteroids modulate cortical development. Then alterations in neurosteroid levels may contribute to abnormal neurodevelopment. These findings suggest that connectivity between the thalamus and cortex is likely altered by neonatal and early postnatal neurosteroids and may results in a disinhibited frontal cortex. It opens possibility to augment low levels with exogenous neuroactive steroids.
- To study metabolomic fingerprinting and profiling focused on membrane neuroactive steroid, lipid peroxidation products from plasma samples and brain region.
- Identification of specific changes in neurosteroid contents during development in an animal model may lead to the identification of the new and highly specific tools for early diagnosis.
- Meta-analysis and correlation of results obtained in the study.
- Augmentation of neuroactive steroids can represent strategy to maintain excitation-inhibition balance of the brain during neurodevelomental processes.

Naturally occurring neurosteroids regulate many functions of the central and peripheral nervous system ranging from the development to complex behavior. The neurosteroids represent a complex cohort of well-balance endogenous compounds. They can modulate the function of membrane receptors for various neurotransmitters, namely $GABA_A$ receptors, NMDA receptors. These mechanisms are likely responsible for their psychopharmacological effects and may account for their antidepressant or antpsychotic effects as well as for their positive influence upon learning and memory functions.

Neurosteroids are involved in several CNS physiological and pathological processes, such as the response to stress, depression, anxiety, schizophrenia, or memory deficit. Antidepressant effects of neuroactive steroids were described in animal models as well as in patients. It has been shown that antidepressant treatment normalized the imbalance of 3α and 3β pregnanolone in patients suffering from depression. During social isolation, an animal model of depression-like behavior, biosynthesis of pregnanolone is significantly decreased. SSRI are able to reverse the decreased brain pregnanolone level, and to correct behavioral deficits. Same, chronic stress reduces the production of allopregnanolone, as well as other GABAergic neurosteroids. Decreased levels of neurosteroids in the plasma and CSF are found in patients with major depression. Prolonged exposure to stress may induce a reduction in pituitary responsiveness to high concentrations of CRH, leading to desensitization, and consequently to decreassed ACTH secretion, affecting neurosteroid synthesis. A reduced ACTH response to chronic stress leads to hyper-responsiveness of the hypothalamic–pituitary–adrenal axis (HPA) axis to the new stimuli, and confers vulnerability to mood and anxiety-related disorders, as well as depression. Antidepressant treatment normalizes the altered allopregnanolone levels suggesting that GABAergic neurotransmission alteration by the neurosteroids has a therapeutic effect

Several lines of evidence suggest that neuroactive steroids may contribute to the pathogenic role of psychosocial stress in schizophrenia also. Psychosocial stress leads to alterations in the levels of circulating neurosteroids. Same, stress causes changes in the synthesis and metabolism of neurosteroids, which in turn play an important role in the neurobiology of the behavioral response to stress. Next, neurosteroids have been shown to play a key role in the regulation of dopaminergic neurotransmission. Finally, schizophrenia patients have been shown to feature abnormalities of neuroactive steroid profiles. And antipsychotics can normalize abnormal neurosteroid levels; moreover, neuroactive steroids also possess antipsychotic potential.

Brain is capable to synthesize neurosteroids already from the embryogenesis period. Later, during development, they regulate dendritic growth, spine generation and synaptogenesis in cerebellum (Sasahara et al., 2007). It has been shown that administration of estradiol promotes dendrite growth and spine formation in Purkinje cells of cerebellum (Sakamoto H. et al., 2003). Additionally, pregnenolone induces microtubule assembly (Marukami et al., 2000). Pregnenolone sulphate was found to enhance expression of neuronal cell adhesion molecules and stimulated proliferation in hippocampus (Mayo et al., 2005). The effect of neurosteroids during the development is mediated not only via receptors for neurotransmitters but also via nuclear receptors (ERα and β) as well as other targets. Recently, transient receptor potential melastatin 3 (TRPM3) channel was discovered to be modulated by the pregnenolone sulphate. Activation of TRPM3 by pregnenolone sulphate potentiates spontaneous glutamate release onto neonatal Purkinje cells during a period of active glutamatergic synapse formation (Zamudio-Bulcock et al., 2011).

Next the action of neurosteroids is mediated also likely by suppression of inflammatory reaction (He J. et al., 2004), providing neurotrophic support by increasing of brain-derived neurotrophic factor (BDNF) levels (Stein and Wright, 2010), reducing lipid peroxidation and oxidative stress (Roof et al., 1997), promoting remyelination (De Nicola et al., 2006) and by preventing excitotoxicity via NMDA receptor inhibition and GABAA receptor potentiation (Weaver et al., 1997).