Department of Women's and Children's Health

Astrid Lindgren Children's Hospital

Karolinska institutet, Stockholm, Sweden

Studies on Neuronal Signaling in the Hippocampus related to Development, Pathogenesis and Treatment of Mood Disorders

Nermin Sourial-Bassillious

Stockholm 2010

Published papers and figures have been reprinted by permission from:
Pediatric research, no. 2536401349151, Oct 26, 2010, Wolters Kluwer Health.
Neuroscience, no. 2536110518052, Oct 25, 2010, Elsevier.
Published by Karolinska Institutet. Printed by [US-AB]

ISBN [978-91-7457-148-6]

"The God of heaven Himself will prosper us; therefore we His servants will arise and build" Nehemiah 2:20

"Nothing can make a human being happier than thinking" (Wise Father)

"Not everything that counts can be counted, and not everything that can be counted counts." Albert Einstein

TO MY PRECIOUS KIDS

(GEORGE, MARY, JOY, JANIE AND JOHN)

Abstract:
The hippocampus is a central organ in the brain which is interconnected with different cortical regions and plays an important role in memory and learning processes. The anatomical position of the hippocampus together with the special sensitivity of its neurons renders it vulnerable to brain injury. This vulnerability is more pronounced during the developmental stages of the hippocampus and more specifically to hypoxic ischemic injuries. Such injuries affect the neuronal circuit formation and the synapses between neurons, which in turn affect the crucial functions of the hippocampus. MHC-I molecule has been found to play a role in the development and function of some neuronal systems in the visual cortex. It is also expressed in the hippocampus and plays a role in the functional plasticity of this organ. Dysregulation of this molecule, by different cytokines released during hypoxic ischemic brain injuries, speculated to be among the reason of hippocampal dysfunction. Recent findings point to the involvement of the hippocampus in the pathology and treatment of several mood disorders. This is more evident in depressive disorders, including major depression disorder and bipolar disorder, which are becoming more recognized among adolescents and children. Selective serotonin reuptake inhibitors and lithium are considered the most reliable in treating such disorders even among children and adolescents. However their use is still debatable due to severe side effects and increased suicidal ideations. Further research providing more information on the possible mechanisms of actions of these pharmacological therapies would lead to a better understanding of the cellular mechanisms behind depressive disorders. Such understanding would help in developing more target specific drug therapies for treating depressive disorders in young patients.
In this study, and first, we provide further evidence for the vulnerability of the hippocampus during the perinatal period. The expression of MHC-I and CD3ζ in the hippocampus is vulnerable during selective periods of development. TNF-α is a factor that would alter the MHC-I/CD3ζ signaling system. Taking into consideration the dual role of MHC-I and CD3ζ molecules as regulators of development and plasticity in the CNS, we hypothesize that alterations in the expression levels of these molecules may be involved in the pathogenesis of neuropsychiatric disorders.
Second, we highlight the fact that down-regulation of glutamate mediated calcium signaling is a potential target for lithium action. Together with previous reports on the hyperactivity of intracellular calcium ion mobilization in the peripheral cells of bipolar patients, one can speculate that calcium hyperactivity may play a role in the pathogenesis of bipolar disorder. Considering the importance of neuronal calcium homeostasis for the normal function of neuronal circuits and synapses, the use of lithium when treating children and youth can have advantages as where neuronal circuits and synapses are in the phase of maturation.
Third, we describe a unique distribution and vesicle trafficking of 5-HT1BRs in the dendrites of hippocampal neurons. This finding sets 5-HT1BRs apart from the majority of postsynaptic receptors and opens a new channel for a receptor-specific approach to 5-HT signal regulation. Using such channel could provide a more target specific anti-depressive therapy that would have more specific action with less side effects, when treating children and adolescents.

LIST OF PUBLICATIONS

I. **Sourial-Bassillious N**, Eklöf AC, Scott L, Aperia A, Zelenin S. Effect of TNF-alpha on CD3-zeta and MHC-I in postnatal rat hippocampus. Pediatr Res. 2006 Oct;60(4):377-81.

II. **Sourial-Bassillious N**, Rydelius PA, Aperia A, Aizman O. Glutamate-mediated calcium signaling: a potential target for lithium action. Neuroscience. 2009 Jul 21;161(4):1126-34.

III. Liebmann T, **Sourial-Bassillious N**, Kruusmägi M, Bondar A, Svenningsson P, Flajolet M, Greengard P, Scott L, Brismar H, Aperia A. A novel form of G protein-coupled receptor neurotransmitter transport from soma to nerve terminal. (Manuscript)

LIST OF ABBREVIATIONS

5-HT	5-hydroxytryptamine (serotonin)
5-HT1BR	5-hydroxytryptamine 1B receptor
ADHD	Attention-Deficit/Hyperactivity Disorder
AMPA	α-amino-3-hydroxyl-5-methyl-4-isoxazole-propionate
BD	Bipolar Disorder
Ca^{2+}	Calcium ion
$[Ca^{2+}]_i$	Intracellular calcium ions
CA	Cornu ammonis
CD3ζ	Cluster of differentiation 3 zeta
CNS	Central nervous system
C.S.F	Cerebro-Spinal Fluid
D	Dopamine
DG	Dentate Gyrus
DHPG	Dihydroxyphenylglycine
EC	Entorhinal Cortex
ER	Endoplasmic Reticulum
GluR	Glutamate Receptor
GPCRs	G protein– coupled receptors
IL-1β	Interleukin-1 beta
INF-γ	Interferon gamma
IP	Intra-Peritoneal
IP3	Inositol Tri riphosphate
LiCl	Lithium Chloride
LGN	Lateral Geniculate Nucleus
LTD	Long Term Depression

LTP	long term potentiation
MDD	Major Depressive Disorders
mGluR	metabotropic Glutamate receptor
MHC-I	Major Histocompatibility Complex class I
MAOIs	Monoamine oxidase inhibitors
NK	Natural killer cells
NMDA	N-methyl-D-aspartic acid
PLC	Phospholipase C
PSD	Post Synaptic Density
RT-PCR	Reverse transcription polymerase chain reaction
SpH	Superecliptic pHluorin
SSRI	Selective Serotonin Reuptake Inhibitors
TCR	T cell receptors
TNF-α	Tumor necrosis factor alpha

1 INTRODUCTION

1.1 HIPPOCAMPUS ANATOMY AND FUNCTION

1.1.1 Gross anatomy

The hippocampus is a horseshoe shaped sheet of neurons placed bilaterally in the medial temporal lobes, and adjacent to the amylgdala *(Bear et al.)*. It has a charecteristic, curved shape that has been matched to the sea horse shape of Greek mythology and the ram's horns of Ammun in Egyptian mythology, given the name Cornu Ammonis (CA) *(Duvernoy, 2005)*. It is seen anatomically as an extension at the edge of the cerebral cortex. It can be identified as a zone where the neocortex narrows to become a single layer of densely packed neurons (*Amaral and Lavenex, 2006)*. This gross structure is well conserved across all mammalian species. The course of the hippocampus follows the medial aspect of the ventral floor of the inferior horn of the lateral ventricle, ending at the temporal pole where it becomes continuous with the fornix below the splenium of the corpus callosum. The cortical region adjacent to the hippocampus is known as the parahippocampal gyrus, it includes the enterorhinal cortex and the perirhinal cortex [Figure 1]. These two regions derive their names from their relation to the rhinal sulcus *(Duvernoy, 2005)*.

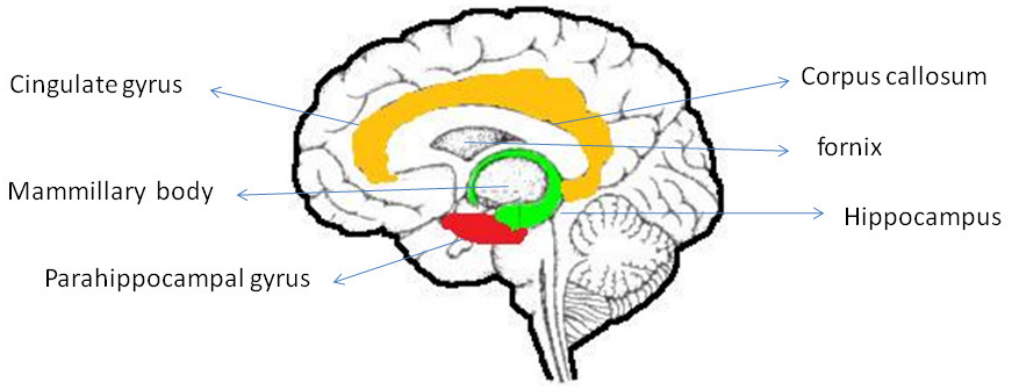


Figure 1:Gross anatomy of the hippocampus. *Figure adapted from Santiago Ramón y Cajal [1909] (1911). Histologie du Systeme Nerveux de l'Homme et des Vertebretes. Paris: A. Maloine*

The brain gets blood supply from the internal carotid artery, which is commonly divided into its intracranial part and its extracranial part. The extracranial part of the carotid artery branches into the middle cerebral, anterior cerebral and posterior cerebral arteries to form the Circle of Willis. Blood supply to the hippocampal region is quite

variable between individuals. The main blood supply to the hippocampus usually arises from the posterior circulation through the posterior cerebral artery via single or multiple middle and posterior hippocampal arteries. The head of the hippocampus is supplied by the anterior hippocampal artery which arises from the anterior choroidal artery *(Thammaroj et al., 2005)*.

1.1.2 Regions and neuronal cell types

The hippocampus is part of the hippocampal formation, which is a complex structure composed of the cornu ammonis (CA), subiculum, dentate gyrus (DG) parahippocampal gyrus, fimbria, and fornix. On the basis of fiber connections and the type of neurons, the hippocampal formation has been subdivided to the hippocampus proper and the DG *(Amaral, 1978)*. The hippocampus proper can also be subdivided into the CA and the subiculum fields, the principal cells in these fields are the pyramidal cells. The CA can be further into 4 fields (named by Lorente de No in 1934) CA1, CA2, CA3 and CA4 *(Graham et al., 2002)*, depending on the appearance of the pyramidal neurons. The DG comprises the granular cell layer. The hippocampus is a region of the brain that is crucial for memory formation; which is supposed to be due to the neuroplasticity phenomena within hippocampal neurons *(Amaral and Witter, 1989, Bayer, 1985)*.

1.1.3 Interconnection with cortical regions

Investigating the anatomy of the hippocampus and its connection to the surrounding cortical areas is an important step in understanding the hippocampal neuroplasticity. The hippocampus is a site of neuroanatomic convergence in the brain. It receives projections from different adjacent cortical areas, which in turn form circuits into the hippocampus. The entorhinal cortex (EC) is the major source of hippocampal input, as well as a target of hippocampal output. The EC regions are also strongly and reciprocally connected with many other parts of the cerebral cortex. The superficial layer of the EC provides the most prominent input to the hippocampus, and the deep layers of the EC receive the most prominent output from the hippocampus *(Suzuki and Amaral, 1994)*. Within the hippocampus, the flow of information is rather unidirectional. Neurons of layers II and III of the cortex projects to the granule cells of the DG. The granule cells in turn project to the large pyramidal cells of the CA3 field. Finally the CA3 pyramidal cells project to the pyramidal cells of the CA1, then back to the cortex [Figure 2] *(Amaral and Insausti 1990, Andersen, 1975)*. The interconnection

between the hippocampus and the adjacent medial temporal cortical areas mediate the memory functions of the hippocampus *(Swanson, 1982)*.

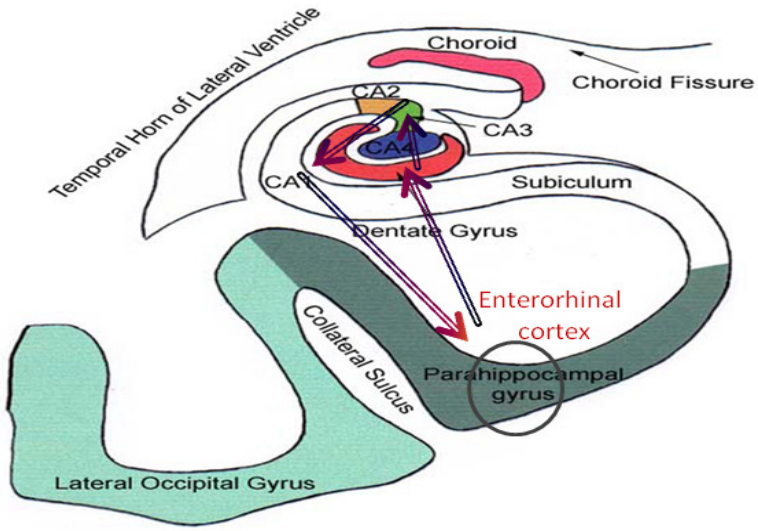


Figure 1: Divisions and interconnections of the hippocampus, showing the neural circuit, starting from the enterorhinal cotrex part of the parahippocamal gyrus. Efferents from the enterorhinal cortex projects to the dentate gyrus. Efferents project from the dentate gyrus to the CA3 area, and finally the pyramidal cells of the CA3 projects to the CA1 area. *Figure adapted from the mesial temporal sclerosis report in the eMedicine Specialties, Radiology section, Brain / spine subsection.*

1.1.4 Role in memory and mood

The hippocampus is well known to play important role in memory. It is essential in the formation of new memories of experienced events as well as in recalling old memories *(Suzuki, 2007)*. The hippocampus is also responsible for long term memory, by which one can store larger quantities of information for potentially unlimited periods of time through stable and permanent changes in the neuronal connections *(Jarrard, 1993)*. This form of neuronal plasticity - known as long term potentiation (LTP) - was first discovered and best studied in the hippocampus. LTP is a long-lasting enhancement in signal transmission between two neurons, which result from synchronous stimulation *(Wittenberg and Tsien, 2002, Behr, 2009)*. This phenomenon is considered to be one of the major neuronal mechanisms that underlie learning and memory function of the hippocampus *(Gruart and Delgado-García, 2007)*.

The idea that the hippocampus is a purely cognitive structure involved only in memory has been challenged during the last 2 decades. Results from extensive research in the molecular basis of depression and depression-related mood disorders have

led to a variety of hypotheses that indicate the involvement of the hippocampus in mood disorders *(Lee et al., 2010).*

Alteration in hippocampus size and disturbances in the hippocampus neurogenesis in mood disorders are among the evidences of the hippocampus involvement in the pathology of mood disorders, particularly in depressive disorders *(Rigucci el al., 2010, Balu and Lucki, 2009, Savits and Drevets, 2009).* Data from preclinical studies suggested that regulation of hippocampal plasticity and neuronal survival may be associated with the therapeutic effects of mood stabilizers *(Frey et al., 2007).* Furthermore, reductions in synapses and synaptic proteins were evident in bipolar disorder (BD) patients in the hippocampal ventral CA1 region (*Eastwood and Harrison, 2000, Rosokilja et al., 2000*).

Chemical neuroimaging studies in BD further implicate altered excitatory glutamate neurotransmission as well as cellular and membrane metabolism, which is especially pronounced within the hippocampus *(Ng et al., 2009).* N-methyl-D-aspartic acid (NMDA) receptors *(Toro and Deakin, 2005, Scarr et al., 2003)* as well as group I metabotropic glutamate receptors (mGluR) *(Pilc et al., 1998, Palucha and Pilc, 2002)* are implicated in depressive disorders, via modifications in the LTP and synaptic plasticity in the hippocampus *(Popoli et al., 2002).* Another neurotransmitter system that has been implicated in the pathophysiology of psychiatric affective disorder is the serotonergic system. Pharmacologic and genetic studies have suggested specifically, a role for 5-hydroxytryptamine receptor 1B (5-HT1B) receptors in the pathophysiology of depression *(Coppen and wood, 1982, Moret and briley, 2000).*

A brief summary: The current information indicates that the hippocampus is a central organ that through its interconnections with adjacent brain regions and the characters of its neurons plays an important role in memory and mood regulation.

1.2 HIPPOCAMPUS DEVELOPMENT AND VULNERABILITY

1.2.1 The course of development

The structure of the human hippocampus is easily recognized at birth *(Arnold and Trojanowski, 1996),* but it undergoes substantial postnatal maturation throughout infant and juvenile development *(Giedd et al., 1996).* The majority of the neurons in the primate hippocampus migrate prenatally *(Eckenhoff and Rakic, 1988, Nowakowski and Rakic, 1981)*, and therefore, the fundamental cytoarchitectonic appearance of the hippocampal subfields is stable after birth. Moreover, there is

progressive neuronal enlargement and a decrease in neuronal density throughout childhood and adulthood *(Humphrey, 1967, Seress, 1998)*.

In the early postnatal period, growth cones start to appear; dendrites grow and become covered with spines *(Lang and Frotscher, 1990)*. The early neuronal development including exuberant outgrowth of axons and the formation of connections with multiple targets is an essential component of a well-functioning nervous system. The axons develop by forming several projections that are later refined through axonal pruning to establish the final connectivity that is maintained into adulthood *(Low and Cheng, 2005, Luo and O'Leary, 2005)*.

Another essential component of a well functioning nervous system are well formed and functioning synapses. Significant synaptic modifications occur during hippocampal maturation. Differences in the developmental profile for synaptophysin expression show that not all the synapses in the hippocampal region have the same levels of maturation, which reflects differential maturation of distinct functional circuits. Moreover, electron microscopic and stereological studies shows selective overproduction of asymmetrical, axospinous synapses during infancy, which then declines towards adulthood *(Eckenhoff and Rakic, 1991, Lavenex et al., 2007)*. Abnormalities of neuronal growth and maturation, and synapses formation might underlie various behavioral disorders. Furthermore, various factors as well as pharmacological treatments can affect these processes.

A brief summary: The concept of developmental neurobiology indicated changes over time including environmental effects which is of great importance for understanding psychopathology and possibly offers new strategies for the treatment of psychiatric disorders.

1.2.2 Selective vulnerability

The hippocampus is one of the most vulnerable areas in the brain, being responsible for learning and memory *(Cai et al., 1999, De Jong et al., 1999)*. Animal studies revealed that the hippocampus, and particularly the CA1 area, was selectively vulnerable to the consequences of hypoperfusion and brain ischemia *(Schmidt-Kastner and Freund, 1991)*. Furthermore, magnetic resonance imaging of children with perinatal hypoxia, revealed lateral ventricular hypertrophy with abnormal intensity of the adjacent white matter in the anatomical position of the hippocampus *(McQuillen and Ferriero, 2004)*. The main reasons behind the selective vulnerability of the hippocampus are the high sensitivity of the pyramidal neurons of the CA1 region, the rather limited arterial blood

supply to the hippocampus, and the anatomical position of the hippocampus, as it is adjacent to the lateral ventricle *(Thammaroj et al., 2005)*.

Studies done on both rodents and primates indicate that perinatal hypoxia alters the normal development of the hippocampus. This early hippocampal insult has significant impact and yields profound losses of context-rich memory abilities later in life. Clinical follow up on human perinatal hypoxic infants reported learning impairment and developmental amnesia in children *(Zola-Morgan and Squire, 1986, Bachevalier et al., 1999, Pascalis and Bachevalier, 1999)*.

Furthermore, in primates, neurogenesis in the hippocampus proper and DG occur almost entirely during prenatal life, in contrast to rodents. However, many morphological and neurochemical changes, together with the refinement of the synaptic connections within the hippocampus, persist into the first postnatal years *(Machado et al., 2002, Seress, 2001, Benes et al., 1994)*. Thus, although the structural items and synaptic connections essential for memory formation are present in the newborn primates, the adaptations of hippocampal circuits from birth to adulthood postulate a basis for hippocampal-dependent memory process to continue to mature during childhood *(Utsunomiya et al., 1999, Nelson, 1995, 1997)*.

1.2.3 Cytokines release in perinatal hypoxia

Perinatal hypoxic-ischemia is an important factor affecting normal development and maturation of the central nervous system (CNS). Studies done on the periventricular white matter in hypoxic ischemic rats revealed damage to the axons and the oligodendrocytes *(Dammann et al., 2001, Ness et al., 2001)*. This is considered to be the reason behind the disruption of the white matter fiber tracts and disturbances of the function of neural networks, leading to neurobehavioural syndromes *(Filley, 2005)* or neurological abnormalities *(Mulhern et al., 2001)*.

The pathogenesis of the damage in perinatal hypoxia is a complex process and not fully understood. Recent studies have shed light on the role of activation of microglia cells in this pathogenesis. The release of proinflammatory cytokines has been implicated in this process *(Kaur and Ling, 2009, Chew et al., 2006)*. Several cytokines are up-regulated in the cerebrospinal fluid (C.S.F) and blood of infants exposed to hypoxic ischemia. Among these cytokines are Interleukin-1 beta (IL-1β), Interleukin-6 (IL-6) and Tumor necrosis factor alpha (TNF-α), all of which are associated with hypoxia-triggered brain damage *(Shalak and Perlman, 2002, Aly et al., 2006, Shohami et al., 1999)*. Several

follow up studies evaluating the brain damage through magnetic resonance imaging, showed that the extent of posthypoxic brain lesions is correlated with elevated levels of IL-1β and TNF-α, in both blood and C.S.F *(Oygur et al., 1998, Bartha et al., 2004, Foster-Barber et al., 2001).*

1.2.4 MHC-I dual role

Major histocompatibility complex class I (MHC-I) molecules are immune molecules that play an important role in the protection against harmful agents, being responsible for antigenic peptide presentation to cytotoxic T lymphocytes. MHC-I molecules are recognized by several families of receptors in the immune system, including T cell receptors (TCRs), natural killer (NK) receptors, and cluster of differentiation 8 (CD8) dimers *(Ugolini and vivier, 2000, Moretta et al., 1997).* Of these, cluster of differentiation 3-zeta (CD3ζ) is a transmembrane glycoprotein, known to couple with TCRs and some NK receptors *(Bakkeret al., 2000, Kane et al., 2000).*

The discovery of MHC-I in the brain has challenged the concept that the brain is an immune privileged organ that express immune molecules only in vitro or under pathological conditions *(Howard and Thompson, 1998, Wekerle, 2002, Syken and shatz, 2003).* MHC-I was initially discovered by Carla Shatz *(Corriveau et al., 1998)* in the brain, through an unbiased differential screening of the mRNA of several proteins, aiming to identify molecules required for activity-dependent refinement of connections during visual system development. Their *in-situ* hybridization and immunohistochemistry studies revealed that the MHC-I and CD3ζ are expressed on the neurons of different brain regions, such as the lateral geniculate nucleus (LGN) and the CA1 area of the hippocampus. Furthermore, they also found that class I mRNA levels were dynamically regulated during the development of the lateral geniculate nucleus. The peak period of expression of class I MHC mRNA in the LGN coincides with the periods of most extensive retinal ganglion axon arbor growth and remodeling *(Sretavan and shatz, 1986a).* This occurs during the prenatal formation of the eye-specific layers *(Shatz, 1983, Sretavan and shatz, 1986b)* and the early postnatal period when ON and Off center retinal ganglion cell axons are known to be prone to activity blockade *(Dubin et al., 1986).* Moreover, in the hippocampus, the MHC-I was found to be expressed at lower levels prenatally, but was subsequently expressed at high levels in the mature neurons. Adult forms of activity-dependent plasticity, such as long-term potentiation (LTP) and long-term depression (LTD), are known to be present in mature neurons *(Malenka, 1994; Crair and Malenka, 1995; Kirkwood and Bear, 1995).* These

observations indicated that MHC-I may play a novel role in neuronal signaling and activity-dependent changes in synaptic connectivity.

Further research in this field using knockout mice indicated that MHC-I function is required for the developmental refinement of the retinal projections. Axonal projections of the reticulogeniculate neurons are altered in mice lacking any of the components of the MHC-I complex. Electrophysiology studies show that the MHC-I/CD3ζ signaling complex is important for mediating activity dependent synaptic plasticity *(Huh et al., 2000, Katz and shatz, 1996, Feldman et al., 1999)*. This supports the role of MHC-I in the refinement of retinal projections, where activity-dependent structural reorganizations during development are thought to arise from cellular mechanisms of synaptic plasticity *(Goodman and shatz, 1993).* MHC-I immunostaining is localized in the postsynaptic densities and dendrites of hippocampus neurons in culture, suggesting a role for MHC-I in the homeostatic regulation of synaptic function and morphology in the hippocampus *(Goddard et al., 2007).* The evidence to date generally supports a model, in which class I MHC functions in the CNS by engaging CD3ζ-containing receptors to signal activity-dependent changes in synaptic strength, ultimately leading to the establishment of appropriate synapses. Class I MHC may act directly at the synapse to assist the elimination of unsuitable connections. This is thought to occur via signaling mechanisms already characterized in immune cells *(van Leeuwen and Samelson, 1999)* such as phosphorylation of CD3ζ by fyn [a kinase previously implicated in hippocampal plasticity] *(Grant et al., 1992)*. However there are no experimental evidences yet to support this hypothesis.

MHC-I and CD3ζ being immune molecules, can be altered by different immune responses, where cytokines are released such as infection and hypoxia. Several in vitro studies have reported that different cytokines such as Interferon gamma (INF-γ) and TNF-α regulate the MHC-I and the CD3-ζ cell surface expression *(Neumann et al., 1997, Isomaki et al., 2001)*. Furthermore, recent *in vivo* studies have now demonstrated a normal role of cytokines such as TNF-α, in LTD *(Beattie et al., 2002)* and *in-vivo* occular dominance plasticity *(Kaneko et al., 2008)*. In contrast increasing evidences suggest that TNF-α can exert destructive effects on CNS cells *(Louis et al., 1993; Talley et al., 1995; Vartanian et al., 1995).*

Thus, damage and inflammation might cause changes in synaptic plasticity and memory function through disturbance in MHC-I expression. It is not yet established whether alterations in the MHC/CD3ζ induced by the different cytokines, is responsible

for the damage coinciding with perinatal hypoxia in the hippocampus. The lack of such evidence essentiate further research in this field.

1.3 HIPPOCAMPUS IN MOOD DISORDERS

1.3.1 Mood disorders

According to the World Health Organization (WHO) *(Murray and Lopez, 2002)*, mental health disorders are one of the leading causes of disability worldwide. Three of the ten leading causes of impairment in people between the ages of 15 and 44 are mental disorders, and the other causes are often accompanied by mental disorders.

Mood disorders such as major depression disorder and BDs are among the most pronounced psychiatric disorders in modern society. About 16% and 1% of the population are estimated to be affected by major depression and BD respectively one or more times during their life time *(Kessler et al., 2005)*. The presence of the common symptoms of these disorders gave them collectively the name 'depressive syndrome'. Both disorders includes a long-lasting depressed mood, feelings of guilt, anxiety, and recurrent thoughts of death and suicidal ideations *(Nestler et al., 2002)*.

Major depression and BD are two related disorders. BD is still supposed to be under-diagnosed primarily due to misdiagnosis as unipolar depression *(Manning, 2010)*. Depressive onset BDs begin earlier than the ones with manic onset, have a longer duration, chronic course with frequent recurrences, a depressive dominant polarity, higher lifetime rate of suicidal behavior, less psychotic symptoms and more rapid cycling *(Besnier et al.,2010)*.

Retrospective and prospective research has shown that most adulthood mental disorders begin in childhood and adolescence *(Kessler et al., 2007)*. However, the symptoms of BD in children and adolescents seem to vary from its presentation in adults. Young people with BD often experience long episodes of mania, along with rage and irritability. Co-morbid disorders in young patients with BD include both Attention-Deficit/Hyperactivity Disorder (ADHD) and anxiety disorders. Early recognition and intervention are crucial *(Chang, 2010)* for improving clinical outcomes and future prognosis.

Untreated early-onset BD is associated with higher rates of rapid cycling, increased co-morbidity, and more severe mania and depression than adult-onset BD. Proper diagnosis of BD early in its course can prevent treating young patient with hazardous

treatments that may exacerbate or worsen the progression of the disorder *(Findling, 2009)*. The use of lithium and SSRIs (selective serotonin reuptake inhibitors) the most commonly used therapies in adult BD and depression - is still debatable in children and adolescents. There is a strong need to develop new therapies with more specificity and fewer side effects that can be used during these vulnerable periods of life.

Extensive research trials are directed towards better understanding of the pathogenesis and treatment of depressive syndromes. Clinical research using different advanced brain imaging techniques, together with genetic studies, shed light on the brain regions and the neuronal systems involved in depressive disorders. Furthermore, Studying the neurobiology and mechanism of action of different pharmacotherapies, would provide better understanding of the possible pathological mechanisms behind the disorders, and provide information on novel specific lines of treatment *(Drevets et al., 2008)*. Most of the studies point to morphological and structural abnormalities in many brain regions, together with disturbances in the neural circuits between them. Several brain neurotransmitters systems including glutamate, serotonin and dopamine have been implicated in depression and mania *(Kalia, 2005)*.

1.3.2 Functional impairment and pathology

Hippocampal and thalamic dysfunction are thought to contribute to the pathogenesis of psychotic conditions such as BD and major depression. BD and major depression are interrelated disorders in the category of depressive syndromes *(Benes et al., 2001, Clinton and Meadow-Woodruff, 2004, Swayze et al., 1992)*.

The hippocampus, located within the medial temporal lobe, contains the DG, CA subfields 1–4 (CA1–CA4) and the subiculum that reciprocally connects to the thalamus, cortical and other subcortical regions *(Friedman et al., 2002, Öngur and Price, 2000)*. Since different brain regions are interconnected via neural circuitry *(Mesulam, 1990)*, it is therefore likely that several regions are affected in mood disorders. Certain changes in the structure, function or chemistry within the hippocampus and thalamus may affect the circuitry involved in emotional regulation *(Adler et al., 2007, Blunberg et al., 2003, Dolan et al., 1990, Strakowski et al., 1999)*. Current neuroimaging studies have provided new insights in the neurobiology of BD and Major Depressive Disorders (MDD), suggesting disturbances in the neuronal circuits to be the pathological factor behind disturbances in the function and structure of the brain and play roles in the development of mood disorders *(Abou-Saleh, 2006, Konarski et al., 2007, Drevets et al., 2008)*.

Changes in the synaptic plasticity, occurring via LTP and LTD, commonly occur in the hippocampus and mediated by ionotropic glutamate receptor (GluR) neurotransmission, empathize the important roles of the hippocampus in cognitive, learning and memory functions *(Bliss and Collingridge, 1993, Law and Deaken, 2001)*. There is a considerable number of studies on the glutamatergic abnormalities of the hippocampus in BD. Several studies on BD patients, have reported disturbances in the ionotropic glutamate NMDA receptor expression and activity within the hippocampus. Furthermore, reductions in hippocampal NMDA receptor subunits NR1/2 and NMDA receptor open ion channel expression have been found in some studies *(Benyto et al., 2007, Law and Deaken, 2001, McCullumsmith et al., 2007, Scarr et al., 2003)*. NMDA receptor dysfunction in the hippocampus and disturbances in the ionotropic (NMDA) glutamatergic intracellular signaling causes disruption in the neural circuits regulating mood *(Clinton and Meadow-Woodruff, 2004, Law and Deaken, 2001)*. It is also well established that the serotonin system is dysfunctional in several disease states, including depression, anxiety, and schizophrenia. It has also been reported that serotonin receptors plays a role in modulating the NMDA receptor function and plasticity in the hippocampus with special implication to major depression disorders *(Bennett, 2010)*.

At present, available structural neuroimaging studies suggest a predominance of detrimental alterations in terms of hippocampal and thalamic volumetric changes in BD, as well as in MDDs. Several recent magnetic resonance imaging (MRI) studies on hippocampal volume abnormalities in depressive disorders, found strong evidence of hippocampal volume reduction in depressed patients, especially in those with repeated depressive episodes *(Videbech and Ravnkilde, 2004)*. These also pointed to the vulnerability of the hippocampus, with volume reductions that may occur before the first clinical manifestation *(references reviewed in Frodl et al., 2008).* Investigators suggest that many factors affect hippocampal volume in patients with MDD. Among these are factors regarding the recurrence, severity and factors intrinsic to individual patients, which appear to be most important *(Drevets et al., 2008, Eker and Gonul, 2009)*. Reduction in the hippocampus, together with other related brain regions, are always found in depressed patients with more persistent forms of MDD (e.g. repeated recurrences, longer illness duration) *(Lorenzetti et al., 2009)*.

Findings from more recent studies of BD patients with properly documented treatment history highlight the possibility that hippocampal volume changes may be related to

treatment. Some studies showed increases in hippocampal volume involved BD patients undergoing treatment with lithium *(Foland et al., 2008)*. Furthermore, a recent 4-year longitudinal study reported increased bilateral hippocampal volumes and improvements in the verbal memory abilities in lithium-treated BD patients. These data suggest that hippocampal changes can occur with treatment over time and may be a marker of neuroprotective effects of lithium *(Yucel et al., 2007)*.

Recent findings concerning antidepressant treatment and clinical outcome, demonstrated that patients who remitted to depression had greater differences in bilateral hippocampal volumes before treatment, compared with non remitters *(MacQueen et al., 2008)*. Other studies showed smaller hippocampal volumes in patients with a depression relapse, than in healthy individuals *(Kronmuller et al., 2008)*. A Swedish study showed increase in the hippocampus volume in depressed patients after electroconvulsive therapies *(Nordanskog et al., 2010).* These results support the assumption that the hippocampus is a pivotal region in the outcome of depression. Results from experimental studies suggest that antidepressants may have an active effect on the hippocampus through neuroplastic processes *(Santarelli et al., 2003)*. These findings have crucial implications on the timing of clinical interventions aimed at reducing the impact of depression on neuronal structure and function *(Mckinnon el al., 2009)*.

1.3.3 Drug target in mood disorders

1.3.3.1 Calcium signaling, glutamate receptors, and lithium

Calcium ion (Ca^{2+}) is the main second messenger that regulates neuronal function, and act as the key carrier of information inside the cell. Changes in intracellular Ca^{2+} concentration is an important and sensitive signaling mechanism and is an essential modulator of synaptic plasticity in the nervous system. The resting intracellular Ca^{2+} concentration is about 0.1microM, whereas the extracellular Ca^{2+} concentration is more than 10000 fold higher. Cytosolic calcium signals originate either from extracellular calcium entering through plasma membrane ion channels or from the release of intracellular stores in the endoplasmic reticulum (ER) via inositol triphosphate (IP3) receptors *(Gleichmann and Mattson, 2010).*

Intracellular calcium ions $[Ca^{2+}]_i$ concentration can be affected by several neurotransmitters, among which glutamate. Glutamate is the major excitatory neurotransmitter in the mammalian CNS and acts through a variety of ionotropic

(ligand-gated cation channels) and metabotropic (G-protein coupled) receptors. The ionotropic receptor group is further subdivided into NMDA, α-amino-3-hydroxyl-5-methyl-4-isoxazole-propionate (AMPA) and Kainate receptors. Metabotropic Glutamate receptors couple via G proteins either to activate phospholipase C (PLC) or inhibit adenylate cyclase activity, according to the type of G protein they are coupled to *(Nakanishi, 1992, Kew and Kemp, 2005)*. Based on the sequence similarities, the six metabotropic receptor subtypes can be further subdivided into three subgroups. This includes group I (mGluR1 and mGluR5), group II (mGluR2 and mGluR3), and group III (mGluR4 and mGluR6) *(Houamed et al., 1991, Tanabe et al., 1992)*. NMDA receptors and group I metabotropic glutamate receptors modulate $[Ca^{2+}]_i$ concentration in the neurons via the two different sources of the ion. NMDA receptors are ion channels that when stimulated by presynaptic release of glutamate induce calcium ions influx from the extracellular space to the neurons *(Mori and Mishina, 1995)*. The group I mGluR couples to the Gq family of heterotrimeric G-protein and PLC proteins. This leads to the triggering of IP3, which binds to specific receptors on the ER and in turn causes the release of intracellular calcium into the cytosol and the activation of protein kinase C (PKC) *(Abe et al., 1992, Masu et al., 1991)*.

Local increase in calcium concentration can result in a number of short-term and long-term synapse-specific alterations, including the insertion or removal of glutamate receptor subunits from the membrane. The changes in the synaptic protein function via posttranslational alterations such as phosphorylation, and the stimulation of the translation or degradation of proteins at the synapse, together lead to changes in synaptic function *(Greer and Greenberg, 2008, Catterall and Few, 2008, Higley and Sabatini, 2008)*. Hyperactivity of $[Ca^{2+}]_i$ mobilization in peripheral cells has been reported in patients with affective disorders *(Dubovsky et al., 1992, Kusurai et al., 1994)*. Therefore, a relationship between the pathophysiology of affective disorders and the abnormality of the intracellular calcium second messenger system has been proposed *(Dubovsky and Frank 1983, Dubovsky et al., 1992)*.

Calcium signaling pathways are hypothesized as targets of modification by long-term lithium treatment. Lithium was introduced into psychiatry almost half a century ago and it still remains the most effective treatment for patients with BD *(Maj, 2000)*. The molecular mechanisms of lithium are still not completely known, however, numerous mechanisms of action have been suggested including regulation of neurogenesis, neuroplasticity and cell death *(Manji et al., 2000)*. Berridge and co-authors suggested

that lithium might act by depleting the cells of inositol, the precursor for IP3, an important stimulator for $[Ca^{2+}]_i$ release *(Berridge et al., 1989)*. Since then, several mood stabilizers have been proven to inhibit Ca^{2+} channels in various neuronal cells *(Kelly et al., 1990, Walden et al., 1992)*, suggesting that Ca^{2+} channel blockers may be useful therapeutic agents for some patients with BD *(Brunet et al., 1990)*. More recently it has been reported that mood stabilizers may modify glutaminergic neurotransmission *(Hough et al., 1996)* and that chronic lithium exposure may attenuate glutamate triggered NMDA receptor-mediated calcium influx *(Nonaka et al., 1998)*.

Lithium is the only mood stabilizer approved by the United States Food and Drug Administration (US FDA) for the treatment of acute mania and BD in adolescents, specifically in ages 12–18 years. Some studies reported the use of lithium in children between 5 and 9 years of age in cases of severe aggressive and explosive behavior *(Cambell et al., 1991)*. The evaluation of lithium use in treatment of BD and severe mood dysregulation in children and adolescents, demonstrated moderate effectiveness of lithium in managing both acute manic and depressive symptoms of pediatric BD. Youths with BD and ADHD co-morbidity have a worse response to lithium therapy than youths with BD only *(reviewd in Hamrin and Iennaco, 2010)*.

Lithium has a narrow therapeutic index, thus can easily become toxic. Lithium toxicity symptoms include loss of balance, vomiting, increased diarrhea, anorexia, ataxia, weakness, blurred vision, polyuria, muscle twitching, coarse tremor, irritability and agitation *(Kowatch et al., 2005)*. Long-term treatment with lithium may also causes nephrogenic diabetes insipidus which results from lithium action on the distal tubules and antidiuretic hormone. These adverse effects may be more commonly observed in younger (5 – 9 years of age), rather than older children *(Cambell et al., 1991)*. Furthermore, lithium can also cause cardiac conduction problems, including atrioventricular blocks and irregular sinus rhythms. It has been reported that younger children may be more likely to develop neurologic adverse effects, including cognitive blunting and headaches, than older children *(Hagino et al., 1995)*.

1.3.3.2 SSRI and serotonin receptors

One of the first neurochemical theories for explaining the pathology of depression was the monoamine deficiency hypothesis *(Prange 1964, Schildkraut 1965)*. This hypothesis has been studied extensively during the last three decades. Studies on the pharmacological and behavioral effects of antidepressant pharmacotherapies in laboratory animals, show that these therapies have prominent actions on

norepinephrine, serotonin and to a lesser extent, on dopamine signaling systems. New modern approaches, including *in vivo* imaging techniques in live patients, together with morphological and neurochemical investigations with high levels of anatomic resolution, implicate multiple system pathology in mood disorders, including abnormalities of monoamines as well as other neurotransmitter systems *(Harro and Oreland, 1996, Mayberg 1997)*.

Serotonin, also known as 5-Hydroxytryptamine (5-HT), is a monoamine neurotransmitter, biochemically derived from tryptophan, and is primarily found in the gastrointestinal tract, and the CNS of humans and animals. In the CNS, the neurons of the raphe nuclei are the principal source of 5-HT release *(Frazer and Hensler, 1999)*. The two major nuclei, from which the majority of brain serotonergic innervations originate, are the dorsal raphe and median raphe nuclei. These nuclei provide an extensive innervation to different brain regions via two separate axonal pathways. For example, the hippocampus is innervated predominantly by the median raphe nuclei. In contrast, the striatum is innervated by the dorsal raphe nuclei. Serotonin is released into the synapses between neurons, and diffuses over a relatively wide gap (>20 µm) to activate 5-HT receptors distributed on the dendrites, cell bodies and presynaptic terminals of the nearby neurons. The widespread innervation of the brain by serotonergic neurons is the anatomic basis for the influence of 5-HT on many diverse brain functions. Serotonergic action is terminated primarily via uptake of 5-HT from the synapses by specific 5-HT monoamine transporter on the presynaptic neurons *(Golden et al., 1992, Heninger et al., 1984)*.

A number of neuroendocrine challenge tests have demonstrated that serotonergic activity is impaired in depressed patients. Repeated treatment of rats with different antidepressants shows that these drugs regulate serotonergic activity by different mechanisms, yet the net effect on enhancing serotonergic transmission is similar *(Blier et al. 1990)*. This effect is regardless of the primary pharmacologic site of action of the drug and includes selective 5-HT reuptake inhibitors, Monoamine oxidase inhibitors (MAOIs), tricyclic antidepressants, and electroconvulsive shock. Selective 5-HT reuptake inhibitors and MAOIs enhance serotonergic transmission by desensitizing the somatodendritic 5-HT autoreceptors *(Blier and de Montigny, 1994)* and enhancing responsiveness of postsynaptic 5-HT receptors *(Haddjeri et al., 1998)*. Long term administration of some tricyclic antidepressants, or a course of electroconvulsive shock to rats, do not appear to desensitize somatodendritic autoreceptors, although these

treatments enhance the responsiveness of postsynaptic 5-HT receptors *(Mongeau et al., 1994)*.

Among the different antidepressant drugs, SSRIs are the most widely used drugs for treating depression and anxiety disorders. The use of antidepressants for the treatment of depressive symptoms in youth has become a common in the clinical practice. However, guidelines from the American Academy of Child and Adolescent Psychiatry together with expert opinion agree that, the use of medication is generally unwarranted unless the depression is severe or recurrent *(Birmaher et al., 2007, Cheung et al., 2008)*. Nevertheless, treatment of all ranges of depressive symptoms with medication has become common practice *(Safer, 1997)*, with SSRIs being the most frequently prescribed drug for mood disorders in children and adolescents *(Ingram and Trenary, 2005)*. Several research studies have found that SSRIs are effective in treating depression in children and adolescents. An evaluation study of the SSRI fluoxetine, reported that with depressed youths between the ages of 7 and 17 years, fluoxetine was much more effective in reducing depression than a placebo *(Emslie et al., 1990)*. On the other hand, there are no conclusive data about the safety of using SSRIs in children and adolescents. Several studies and reviews pointed to the controversy in the idea that SSRI treatment may be associated with increased risk of self-harm and suicidal ideation. It is generally agreed that the appropriate use of SSRIs in children and adolescents requires careful diagnostic assessment, evaluation of co-occurring conditions, and diligent monitoring *(Schahill et al., 2005, Hamrin and Schahill, 2005, Murphy et al., 2008, Cohen 2007)*. A comparative study aiming to report the frequency of common treatment-emergent adverse events from SSRIs in children, adolescents, and adults, reported that children are particularly vulnerable to specific adverse events from SSRIs *(Safer and Zito, 2006)*.

The finding that postsynaptic receptors are targets for antidepressant treatment empathized the role of these receptors in neuronal function regulation. Among the serotonin receptors implicated in depressive disorders are serotonin 1A (5-HT1A), 1B (5-HT1B), 2A (5-HT2A), 2C (5-HT2C), 4 (5-HT4), 6 (5-HT6) and 7 (5-HT7) *(Navines et al., 2008, Kato et al., 2009, Svenningsson et al., 2006, Shaikh et al., 2008, Benedetti et al., 2008, Rosenzweig-Lipson et al., 2007, Warner-Schmidt et al, 2009, Svenningsson et al., 2007, Mnie-Filali et al., 2007)*. Postsynaptic receptor trafficking in the dendrites has recently appeared to be an important pathway in the regulation of neuronal function. It appears to determine the efficiency of the neurotransmission response,

adjust the neuronal pathway specificity, regulate receptor sensitization and control signal plasticity *(Renner et al., 2008, Lee et al., 2008, Petrini et al., 2009, Heine et al., 2008, Park et al., 2004, Makino and Malinow, 2009)*. Such causal factors of dendritic receptor expression and organization have become a crucial target for novel neuropharmacological therapy research *(Fumagalli et al., 2008, Kristiansen et al., 2010, Marchese et al., 2007)*.

Membrane receptors are described to undergo recycling upon activation via endo- and exocytosis, both within and outside of the synapse *(Park et al, 2004, Yudowski et al., 2007)*. Lateral diffusion of receptors is a new concept that describes passive Brownian-like transport of proteins in the neuronal membranes, driven solely by the available thermal energy. This energy-efficient trafficking, illustrated by population and single molecule mobility studies, play a crucial role in the responsiveness to neurotransmitter release by tuning the availability of receptors at the synapse *(Ashby et al., 2006, Jacob et al., 2008, Thriller and Choquet, 2003, 2005, Choquet and Thriller, 2003)*. Many neurotransmitter receptors are now known to undergo recycling and lateral diffusion for effective and efficient response modulation *(Cognet et al., 2006, Makino and Malino, 2009, Petrini et al., 2009)*. Changes in both lateral diffusion and receptor recycling rates are a principle means by which postsynaptic receptors alter neuroplasticity *(Makino and Malino, 2009, Petrini et al., 2009)*. Better understanding of such regulation mechanisms of the synaptic neuroplasticity and the receptors that might have specific modes of mobility, opens the door towards finding new specific targets for antidepressant pharmacotherapies, with less side-effects than other conventional antidepressants.

2 AIMS OF THE STUDY

The overall aim of this study is to elucidate the potential vulnerability of the hippocampus in the pathogenesis and treatment of neuropsychiatric disorder. Further, to study the expression and function of some of the receptors implicated to play a role in the hippocampus development and function in relation to mood disorders.

The specific aims of the study are:

1. To further elucidate the vulnerability of the hippocampus during certain developmental periods, using an immunological model that was recently found to play a role in the hippocampus neuronal Function.
2. To get a better understanding of the mechanism of action of lithium, a commonly used drug in treating bipolar disorder, aiming to find a more target specific therapy.
3. To highlight some novel aspects of some serotonin receptors and to study their mobility. This could provide a potential target for new anti-depression therapy.

3 EXPERIMENTAL PROCEDURES

For detailed description of all materials and methods used in this study, please refer to the original papers enclosed. Here, is a general outline of the methods used.

3.1 MATERIALS

Hippocampus tissue extracted from Sprague Dawley rats at different ages was mainly used to prepare lysates for protein/RNA extraction or for primary neuronal cultures. All experiments were approved by the local committee on Ethics for Animal Experimentation, Stockholm, Sweden.

For the work on MHC-I, animals were injected with Recombinant rat TNF-α and recombinant rat IL-1β, that were obtained from R&D Systems, UK. The study was performed on early postnatal (3-8 postnatal days, EPN), weanling (18-25 postnatal days) and adolescent (38-45 postnatal days) rats. Each group was divided into three subgroups composed of animals of equivalent body weight. Two subgroups were injected with cytokines and one group was injected with vehicle (control). Each cytokine was reconstituted in phosphate buffer saline (PBS) and injected intra-peritoneal (IP); 1 μg for the EPN group, 2 μg for the weanling group and 5 μg for the adolescent group. These doses were selected since they have been shown to have effect on rat brain when injected IP (*Anforth et al., 1998, Kubota et al., 2001)*. The serum level of TNF-α was found to be 1.2 $\pm$ 0.9 pg/mL 24 h after the last injection. Four days after the last injection all animals were anesthetized and the brains were dissected after saline perfusion. Hippocampus tissues were extracted, frozen immediately in dry ice and was later used in all the experiments. We chooses to terminate the experiment after four days since it is well documented that this is the peak period for brain insult following injury, such as hypoxia *(Von Gertten et al., 2005)*. Furthermore, it has been shown that intraperitoneal cytokine Interferon gamma injection has a maximal effect on MHC-I expression at four days *(Xu and Ling, 1994)*. Control and cytokine treated animals had the same weight gain.

3.2 EXPERIMENTAL METHODS

3.2.1 Preparing primary hippocampus neuronal cultures

Hippocampal cell culture is a commonly used model system for addressing a wide range of questions in molecular and cellular neurobiology. Cultured hippocampal neurons pass through defined stages of maturation *(Dotti et al., 1988)* and interconnect

with each other *(Bartlett and Banker, 1984, Fletcher et al., 1994).* Moreover, they are characterized by morphological features *(Banker and Waxman, 1988)* together with the specific expression pattern and localization of neuronal proteins in vitro, which appear to be essentially identical to those of neurons that develop within the intact brain *(Gerrow et al., 2006).* Since the pioneering work of Banker and Cowan *(Banker and Cowan, 1977, 1979)* who established the hippocampal culture system and initially characterized hippocampal neurons and glia, the method and techniques have not significantly changed *(Kaech and Banker, 2006)*, rather some modifications in the medium is used to change the percentage between neurons and glia.

We modified two previously reported protocols to focus more on the neurons in our second and third studies. In the second study we modified the protocol described by Mao and Wang *(Mao and Wang, 2003),* where cytosine-D-arabinofuranoside (AraC) was used for 24 hours to suppress glial growth. In the third study we used another protocol described by Kaech and Banker in 2006, where the medium was modified to nourish neurons and suppress glia. Primary hippocampus neuron cultures have been used in studies 2 and 3 for Calcium imaging, receptor biotinylation as well as receptor mobility and localization experiments.

3.2.2 Studying mRNA levels using real time PCR

Reverse transcription polymerase chain reaction (RT-PCR) is a variant of polymerase chain reaction (PCR). This laboratory technique is commonly used in molecular biology to generate many copies of a DNA sequence, with a process called "amplification". In RT-PCR, an RNA strand is first reverse transcribed into its DNA complement (*cDNA*) using the enzyme reverse transcriptase, and then the resulting cDNA is amplified using traditional or real-time PCR. Real time PCR allow the scientist to follow and view the increase in the amount of DNA as it is amplified. RT-PCR is the most sensitive technique for mRNA detection and quantification, detecting even small changes.

In the beginning of the first study we quantified the MHC-I with the traditional PCR technique. After preparing the cDNA, PCR mixture was added, which contain a standard PCR buffer (Promega, Madison, WI, USA), 2,5 mM $MgCl_2$, 0,2 mM dNTP (Roche, Indianapolis, USA), 22 ρmol of each of the MHC-I primers, 44 ρmol Classic II Primers (with a ratio of 2:1, Classic pair to competimer) (Ambion, TX, USA), and 12 U of AmpliTaq Gold (Perkin-Elmer, Foster City, CA, USA). The PCR mixtures, containing the cDNA, were divided into 5 reactions of 22 μl each, and subsequently

amplified in 25, 26, 27, 28 and 29 cycles, according to the following protocol [1) 7minutes of denaturation at 95°C; 2) 29 cycles of, 30 sec denaturation at 94°C, 30 sec of annealing at 60°C, and 1minute of extension at 72°C; and 3) a final extension step of 5 minutes at 75°C. The thermocycler was paused after 25, 26, 27, 28 cycles, and samples were kept at 65°C for ten minutes]. The PCR products were run on 2% agarose gel with 1x TAE buffer, containing 1x concentration of Gel star Nucleic Acid Gel Stain (Cambrex Bio Science Rockland,U.S.A). GeneRuler 100 bp ladder (Fermentas, Vilnius, Lithuania) was used for sizing of PCR fragments. Digital images were acquired with use of a Flour-S MultiImager and analyzed with the original software Quantity One, version 4.2.1 (Bio-Rad Laboratories, Hercules, CA, USA). The MHC class I primers were selected from different exons to avoid amplification of genomic DNA. The classic II 18S Internal Standards (Ambion, TX, U.S.A) primer set was used as internal control, according to manufacturer's protocol. The size of the 18S PCR fragment is 324 bp, whereas the MHC class I PCR fragment is 223 bp. Through this method, the MHC-I was semi-quantified using the ratio between MHC-I and 18s.

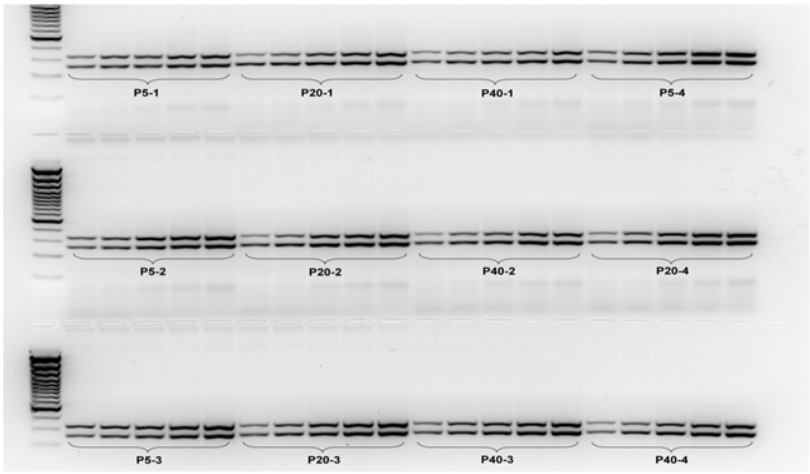


Figure 3. Agarose gel analysis of MHC-I mRNA expression in P5, P20 and P40 rat hippocampus. Left side lane shows the multiple bands of the GeneRuler 100 bp DNA Ladder Plus (Fermentas, Vilnius, Lithuania). Upper band in each lane corresponds to the 324 bp PCR fragment of 18s RNA (the internal positive control) while the lower band corresponds to the 223 bp fragment of the rat MHC-I mRNA. Lanes P5-(1-4) stand for 4 different samples of P5 hippocampus, P20-(1-4) stand for 4 different samples of P20 hippocampus and P40-(1-4) stand for 4 different samples of P40 hippocampus. RT PCR fragments from cycles 25 to 29 for each RNA sample were analyzed.

Agarose gel results are obtained from the end point of the PCR reaction which is time-consuming process. These results are based on size differentiation, and therefore may not be very precise. Furthermore, the end point product varies from one sample

to another. Agaros gels may not be able to resolve variations of 10-fold in yield, where gel resolution is usually very poor. To resolve these problems we moved to Real-Time PCR as it is a more sensitive way to detect changes than Agaros gels, and detecting as little as a two-fold change.

RT PCR and real-time detection of PCR product accumulation was performed using *iCycler* (Bio-Rad). Primers for real-time RT PCR experiments were designed by PrimerSelect software (DNASTAR Inc, Madison, USA) and rat MHC-I mRNA structure (accession number NM_012645) was used to select MHCI- specific primers *(Rada et al., 1990)*. Quantum RNA 18S Internal Standards (Ambion) were used as the "housekeeping" gene, where 18S stands for a ribosomal 18S RNA. This was used to normalize for variations in RNA quality and starting quantity, as well as random tube-to-tube variation in RT and PCR reactions. In our experience, commonly used "invariant" standard controls, such as β-actin, might varied from tissue to tissue, between cell types, or in response to the experimental treatments. The use of ribosomal 18S RNA, as endogenous control, overcome all these problems since the majority of RNA is rRNA.

3.2.3 Studying protein expression, localization and interaction using western blot and immunostaining

The Western blot is an analytical technique used to detect specific proteins in a prepared sample of tissue homogenate or cell extract. Proteins whether native or denatured, are separated according to their length of polypeptides by gel electrophoresis. The proteins are then transferred to a nitrocellulose or PVDF membrane, where they are probed using antibodies specific to the target protein. The proteins in the cell have different locations membranous, cytosolic or nuclear. The use of specific combinations of buffers, detergents and centrifugation speeds determines the different cellular fractions. Other related techniques that include using antibodies to detect proteins in tissues and cells by immunostaining . Antibodies are also used to study protein-protein interaction known by co-immunoprecipitation followed by western blot.

In this study we used different antibodies for western blot, immunostaining and co-immunoprecipitation. An important step before proceeding with any antibody is to test its specificity. Moreover, the size of the band in the western blot should be comparable to the size of the target protein. The size of the protein might vary depending on several factors such as glycosylation or dimerisation.

In the first study we used antibodies against MHC-I and CD3ζ. As for the MHC-I, blotting with OX-18 resulted in two distinct bands, corresponding to 37 and 53-kD, in the lane loaded with whole cell lysates of weanling rats' hippocampus. By using different buffers and centrifugation speeds we prepared cell membrane preparation of the hippocampus the same age rats, to compare the MHC-I protein expression in the total cell lysates and in the cell membranes preparations. The cell membrane preparations were enriched with 53-kD species similar in size with MHC-I protein found by Corriveau in membrane fractions prepared using brain tissues *(Corriveau et al., 1998)*. MHC-I genes are divided in two subgroups, the classical and non-classical classes of MHC-I. Both types of MHC-I are expressed as soluble, as well as membrane-bound proteins. It has also been shown that an intact MHC-I molecule with size 37– 45 kD on SDS polyacrylamide gel may form aggregates with high molecular weight *(Lau et al., 2003)*. The two distinct bands disappeared when omitting the primary antibody. As for CD3ζ, Immunoblot analysis of hippocampal homogenates revealed a band at approximately 37 kD, which corresponds to CD3-z dimers. The CD3-ζ chain is known to form disulphide-linked dimers in SDS polyacrylamide gel, where the two monomers associate via their transmembrane domains. These proteins share the ability to form disulfide linked dimers with themselves and with other members of the family *(Rutledge et al., 1992)*.

In the second study, we used the biotinylation technique to extract neuronal cell membrane proteins. The neurons were first labeled with EZ-Link® Sulfo-NHS-SS-Biotin, a thiol cleavable amine-reactive biotinylation reagent. Cells were subsequently lysed with a mild detergent and the labeled proteins were then isolated with immobilized Streptavidin, 6% beaded agarose. The bound proteins were released by incubating with SDS-PAGE sample buffer and separated by gel electrophoresis. We then used antibodies against NMDA receptor 1 and mGluR5. As with the NMDA blots, we got a single band at the expected size of ~100kD, that disappeared when omitting the primary antibody. For mGuR5, we got a band at ~130 with the cell surface proteins blots, whereas we got a faster migrating band with the neuronal lysate blots.

The specificity of the antibody was tested using HEK (Human Embryonic kidney) cell line T293 which is known not to express mGluR5. The same cell line was transfected with two different mGluR5 constructs, one of which is tagged with Venus and the other untagged. Total protein lysate of the different cell culture plates were

prepared and analyzed by 6% SDS-PAGE. The first lane was loaded with the total protein lysate of the native HEK cells. The second lane was loaded with the total protein lysate of the untagged mGluR5 transfected HEK cells, the third was loaded with the total protein lysate of the Venus-tagged mGluR5 transfected HEK cells and finally the forth was loaded with the total protein lysate of the Venus-transfected HEK cells. No bands were detected in the native HEK cells nor the Venus-transfected cells lanes, while a band of ~130kDa was detected in the untagged HEK cells lanes and a ~150 kDa band was detected in the Venus tagged mGluR5 transfected HEK cells as shown in figure 4.

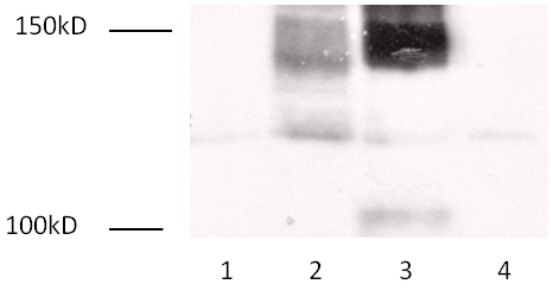


Figure 4: western immunoblotting for mGluR5 in transfected and non transfected HEK cells:
Lane1:HEK cells only
Lane2:HEK cells stably transfected with mGluR5
Lane3:HEK cells transfected with Venus-tagged mGluR5
Lane4:HEK cells transfected with Venus only

With regards to the difference in size between the cell surface protein and the lysate protein, we expected glycosylation and performed deglycosylation experiment. Membrane preparation from hippocampus was produced as previously described (Neuron, Vol.21, 505-520, September, 1998). The protein concentration was adjusted using Bradford. Deglycosylation was performed with PNGase F enzyme kit (New England Biolabs) with some modification to the manufacturers' protocol. Briefly, 20µg protein was mixed with 1µl of 10x glycoprotein denaturing buffer in a volume of 10 µl, and the protein was allowed to denature for 10 minutes at 100^{o}C. NP40 and G7 buffers were added together with the deglycosylation enzyme and incubated over-night at 37^{o}C. Laemmli buffer was added and the samples were heated for 15 minutes at 80^{O}C. Protein extracts were analyzed through 6% sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE). After immunoblotting on nitrocellulose membranes, proteins were probed with the anti-body used to recognize

mGluR5. Deglycosylation caused mobility shift of the protein band as seen in figure 5.

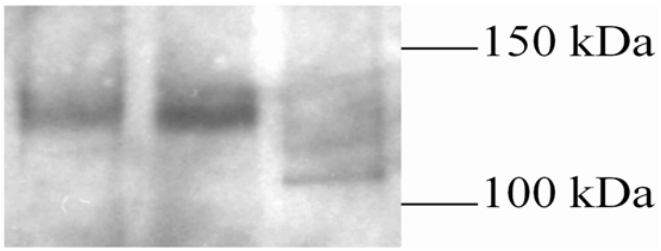


Figure 5: Immunoblots of membranous mGluR5 receptor (20mg protein/ lane) was probed with mGLuR5 antibody. The first lane shows the membranous protein after adding the denaturing buffer, the second lane shows the native ordinary membranous preparation of the protein and the third lane shows the membranous protein after adding the deglycosylation enzyme. Deglycosylation caused mobility shift of the protein band

In the third study, two antibodies against 5-HT1BR, raised against different epitopes were used, according to the required experiment. One was used for western blot, where we prepared different cell fractions using specific buffers and centrifugation speeds. Another antibody was used together with antibodies against Gephyrin, post synaptic density (PSD-95) and Homer for co-immunoprecipitation. This antibody was also used for immunostaining of 5-HT1BR in the hippocampal neurons.

3.2.4 Studying receptor protein localization and movement using confocal microscopy

In the third study we transfected immune-florescent 5-HT1BRs in primary hippocampus neurons to study the localization and movement of this receptor. Confocal microscopy is an optical imaging technique with increased optical resolution and contrast of a micrograph. This is done through using point illumination and a spatial pinhole to eliminate out-of-focus light in thick specimens that are thicker than the focal plane. It allows the reconstruction of three-dimensional structures from the obtained images. This technique has become more popularly used in life sciences for studying of the ultra-structure of cells, more particularly neurons.

Confocal imaging in our study was performed on an inverted Zeiss LSM 510 microscope with 40X (1.2 NA, water) or 63X (1.4 NA, oil) objectives. Emission was induced with laser lines at 458nm (WHAT), 488nm (argon) and 514nm (argon) for cyan, green and yellow labels, respectively. The Confocal imaging was used for studying the localization of the 5-HT1BR and for studying the receptor mobility and

recruitment. Furthermore, the receptor mobility was investigated by performing Fluorescence recovery after photobleaching (FRAP)

3.2.4.1 Fluorescence Recovery After Photobleaching (FRAP)

FRAP is an optical technique capable of quantifying the two dimensional lateral diffusion of a molecularly thin film containing fluorescently labeled probes. This technique is suitable for the analysis of receptor populations' transport of in the dendrites. We expressed fluorescently labeled different G protein coupled receptors including 5-HT1B, 5-HT1B, 5-HT4, dopamine 1 (D1), D2, D5 and mGluR5 in hippocampal cultures. Therefore, FRAP recordings was utilized, where an area of the dendrites was chosen to be bleach and the recovery of the signals from a certain line (axial position) was recorded after bleaching (figure 6a), to detect the movement of the receptors. Furthermore, we choose a small region to detect any intense signals. For all the receptors known to move by lateral diffusion, there is usually increase in the fluorescence that is consistently slow and diffuse indicating gradual population recovery in the bleached regions. Taking D1R as an example for this group we can see in figure 6b, a representative kymograph with gradual population recovery in the bleached region. In addition, FRAP curves for D1R receptor in figure 6c, show gradual population recovery in the bleached regions (green curves), with no striking intensity changes in the small region recordings (blue curves) other than small fluctuating around the full bleached region intensity.

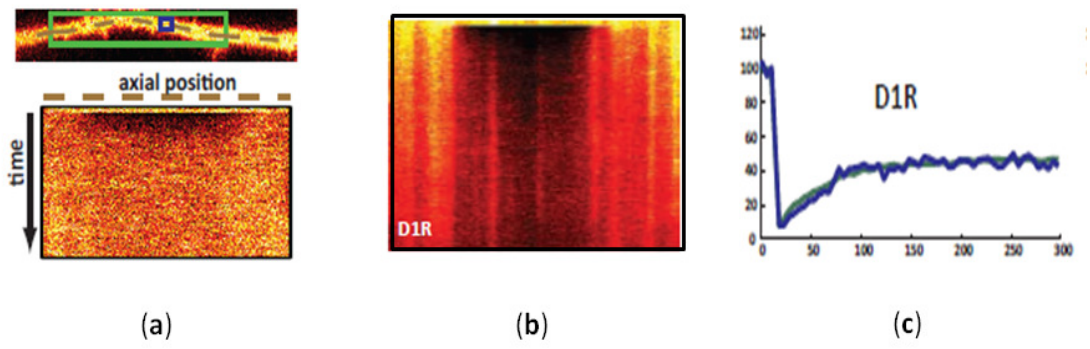
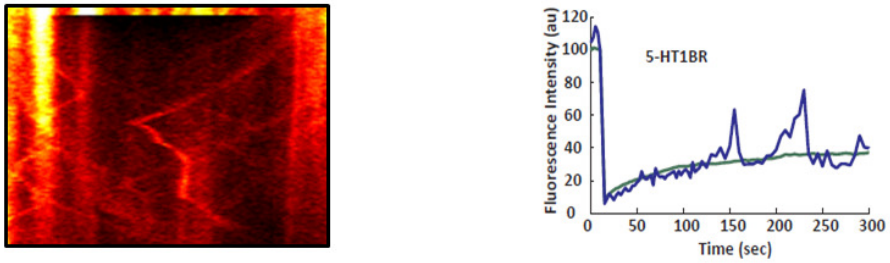


Figure 6: kymograph discritption: (**a**) Top: A model of analysis for FRAP recordings in neuronal dendrites. Green and blue boxes indicate bleaching region (10µm length) and small recovery analysis region (1µm^2), respectively. The brown dotted line represents an example analysis line for kymograph representation. Bottom: Kymograph representation of the model dendrite. Position and time are depicted along the x-axis and y-axis, respectively. (**b**) Kymograph representations of on of the typical G-prtoein coupled receptors, the D1R, FRAP recordings. (**c**) Representative FRAP intensity curves for D1R FRAP recordings.

As for 5-HT1BR, FRAP recordings indicate that there are two distinct modes of 5-HT1BR transport: intracellular aggregate trafficking and lateral membrane diffusion (figure 7a). Aggregate movement on a kymograph appears as slanted lines, whereas immobile aggregates appear as vertical lines. Discrete internal receptor aggregates appear to move with a constant speed, as indicated by nearly constant trajectory slopes. Gradual recovery of background fluorescence also indicates lateral diffusion of receptors. Both modes of transport are seen in FRAP intensity curves in figure 7b. Recovery curves of the full bleached region (green) shows slow population diffusion of receptors in the membrane. Intensity curves of a smaller analysis region (blue) emphasize the transport of internal aggregates.



(a) (b)

Figure 7: 5-HT1BRs exhibit bimodal transport (**a**) Kymograph showing 5-HT1BR recovery after bleaching of a 10µm dendrite fragment on a primary hippocampal neuron. Trafficking of vesicles is seen as diagonal lines. (**b**) FRAP intensity curves of a representative 5-HT1BR recording. The curves represent recovery of the full bleached region (green) and a localized region within the bleached dendrite shaft (blue). Intensity peaks in the blue curve result from vesicle transport through the recorded region.

3.2.5 Imaging of intracellular $[Ca^{2+}]_i$ concentration

In our second study, it was crucial to quantitatively measure the free cytosolic Ca^{2+} concentration, $[Ca^{2+}]_i$, and compare it in response to different stimuli. We used the calcium sensitive Fura-2AM, which is highly selective calcium dual-excitation (ratiometrc) fluorophore, which shifts wave length upon binding to Ca^{2+}. This makes it more suitable for long time measurements because this character makes it suitable for measuring changeable calcium levels without the known problems associated with bleaching and leakage of fluorophore. Moreover, this fluorescent dye was easily loaded into the intact primary hippocampus neurons by incubating them with a membrane-permeant ester derivative. Cytosolic esterases split off the ester groups and leave the membrane-impermeant dye trapped in the cytosol. Since fluorophores are artificial

substances, that they can disturb normal cellular function or even be toxic for the neurons. Several control experiments were undertaken to determine the appropriate concentration and loading conditions.

4 RESULTS AND DISCUSSION

4.1 EXPRESSION AND REGULATION OF MHC-I AND CD3-ζ (PAPER I)

There is increasing evidence of the immune involvement in some neuropsychiatric disorders such as schizophrenia and autism, where the role of cytokines in the immune dysregulation in the brain has been speculated. In this paper we studied the expression pattern of MHC-I and CD3ζ in the rat hippocampus. Moreover we investigated the effect of TNF-α on the expression pattern of these two proteins.

4.1.1 Developmental pattern of MHC-I and CD3ζ in rat hippocampus

We studied the developmental pattern of MHC-I and CD3ζ in the hippocampus of early postnatal (EPN), weanling and adolescent rats. The abundance of the proteins was measured using western blotting of total protein homogenates of the hippocampus. The relative level of CD3ζ protein abundance increased two fold from the EPN to the weanling period, while there was no significant difference between the weanling and the adolescent periods. The MHC-I immunoreactivity in the total protein homogenates yield two bands, corresponding to the soluble and the membrane-bound forms of the protein. The relative level of the membrane-bound MHC-I abundance was significantly lower in the hippocampus of the EPN rats, than in the weanling and adolescent rats. This increase in the MHC-I protein level was parallel to the robust increase found in the MHC-I mRNA, measured by real time PCR, in the hippocampus of rats between the EPN period and the end of the weanling period. No difference was seen in the mRNA levels or in the protein expression level of MHC-I between the weanling and the adolescent rats.

In situ hybridization studies have previously indicated that the expression of MHC-I increases postnatally in the hippocampus *(Corriveau et al., 1998)*. Emerging evidence suggests that MHC-I and CD3ζ play important roles, not only as defense molecules in the immune system but also as regulators of development and plasticity in the CNS. Studies performed on knockout mice have shown that MHC-I and CD3ζ are important for activity-driven structural remodeling and synaptic plasticity *(Huh et al., 2000)*. Important developmental events take place in the hippocampus during the first three weeks of age *(Rice and Barone 2000)*. In the early postnatal period, growth cones start to appear; dendrites grow and become covered with spines *(Lang and Frotscher 1990)*.

Synapse formation and modification is a crucial step in the maturation of the hippocampus, as it is essential for proper circuit formation and function of the nervous system *(Lavenex et al., 2007).*

4.1.2 Effect of TNF-α on CD3ζ and MHC-I expression

We studied the effect of intraperitoneal injections of TNF-α and IL-.1β on the protein expression of CD3ζ and the mRNA expression of MHC-I in the hippocampus of EPN, weanling and adolescent rats. TNF-α caused significant reduction in the protein expression of CD3ζ and in the mRNA expression of MHC-I in the EPN group of rats. In the weanling and adolescent groups, TNF-α had no significant effects neither on the protein expression of CD3ζ nor on the mRNA level of the MHC-I. IL-1β did not have a significant effect on the MHC-I mRNA or the CD3ζ protein levels in rat hippocampus of any age groups.

Perinatal hypoxia is a major cause of neurologic and intellectual impairment in children *(Belet et al., 2004, Vargha-Khadem et al., 2003),* where the hippocampus is highly vulnerable to the consequences of brain hypoxia *(Schmidt-Kastner and Freund, 1991*). Furthermore, cytokines have been implicated in the cause of hypoxia-triggered brain damage *(Aly et al*, *Oygur et al., 1998*). The extent of post hypoxic brain lesions, as evaluated with magnetic resonance images, has been found to be paralleled by an increase in blood levels of several cytokines *(Bartha et al., 2004)*, including TNF-α (*Aly et al., 2006*, *Shohami et al., 1999*, *Foster-Barber et al., 2001).* The regulatory effect of TNF-α on CD3-ζ protein is similar to that found in T-cell hybridoma treated with TNF-α *(Isomaki et al., 2001).* The effect of cytokines on the expression of CD3-ζ in the brain has not been studied to date. It is also reported that TNF-α can alter MHC-I cell surface expression on neurons *(Neumann et al., 1997)*. Moreover there is evidence that TNF-α is a silencer of survival signals in neurons *(Venters et al., 2000).*We propose a specific role for TNF-α in down regulating MHC-I and CD3ζ expression during the period immediately following birth. The down-regulation of CD3-ζ and MHC-I in response to circulating TNF-α might have adverse effects on neuronal development and plasticity. Thus, hippocampus damage induced by perinatal hypoxia may lead to changes in synaptic plasticity via dysregulation of MHC-I expression.

Recent genome-wide studies of large populations reported polygenic variations of human chromosome 6p22.1 at the MHC-I locus are implicated in schizophrenia and

BD *(Shi et al., 2009, Stefansson et al., 2009)*. While many common gene variants in the MHC-I region are strongly associated with schizophrenia and BD, there was no association with several non-psychiatric disorders.

It has also been reported that there is a link between schizophrenia and infection *(Patterson, 2009)*, but the mechanism by which early infection or autoimmune disorders can change brain circuits and behavior is still questionable. Furthermore, it is strongly suggested l that neuronal MHC-I function at the synapses is the link where the immune system would have a variety of direct ways of altering activity dependent synaptic plasticity and neuronal circuits tuning communications *(Shatz 2009)*.

4.2 CALCIUM SIGNALING; A POTENTIAL TARGET FOR LITHIUM ACTION (PAPER II)

It has been postulated for many years that disturbances in calcium signaling is one the mechanism by which lithium can employ its action in mood disorders and other neuropsychiatric diseases *(Wasserman et al., 2004; Perova et al., 2007; Bauer et al., 2003)*. Nevertheless, there are only a few number of studies that have examined the effect of lithium on the calcium signaling pathways in neurons. In this paper we investigated the effect of seven days treatment with lithium on the intracellular calcium signaling, induced by different pathways in the primary hippocampus pyramidal neurons. Furthermore, we studied whether changes in the glutamate receptors are expected to modulate intracellular calcium ions concentration.

4.2.1 Lithium effect on glutamate mediated calcium signaling

The effect of glutamate on $[Ca^{2+}]_i$ was studied in primary cultures of rat hippocampal neurons in the presence and absence of 1mM lithium chloride (LiCl). When control untreated cells were exposed to glutamate (1 mM), a simultaneous increase in $[Ca^{2+}]_i$ was observed in virtually all neurons. This increase was sustained as long as glutamate was present in the solution. Glutamate can increase $[Ca^{2+}]_i$ via activation of ionotropic NMDA receptors and via activation of the group 1 metabotropic glutamate receptors. Specific agonists for each receptor were used to differentiate the different pathways by which glutamate increases $[Ca^{2+}]_i$. In cells exposed to NMDA (10 mM), a sustained increase of $[Ca^{2+}]_i$ was observed, whereas a transient increase of $[Ca^{2+}]_i$ was observed in cells exposed to Dihydroxyphenylglycine (DHPG) (100 mM), a selective group I mGluR agonist. Cells treated with glutamate in the presence of NMDA receptor inhibitor, MK-801 (50 mM), displayed a transient increase of $[Ca^{2+}]_i$, similar to the

DHPG response. Pretreatment of glutamate-exposed hippocampal neurons with LiCl (1 mM) for seven days, resulted also in a sustained increase of $[Ca^{2+}]_i$, however, the maximum amplitude of this response was significantly lower than that observed in non-treated cells. The response amplitude in cells treated with lithium to NMDA and DHPG was also significantly lower than that of non-treated cells.

NMDA receptors are localized at postsynaptic densities (PSDs) where they are structurally organized *(Scannevin and Huganir, 2000)*. They are not simply tightly locked into PSDs, rather, are in rapid equilibrium movement between the neuronal membrane and the intracellular compartment *(Carroll and Zukin, 2000, Nong et al., 2004)*. The group I mGluR receptors, consisting of mGluR1 and mGluR5, are coupled to the Gq family heterotrimeric G-proteinand PLC *(Masu et al., 1991)*. CA1 hippocampal pyramidal cells strongly express mGluR5 that is densely concentrated at the perisynaptic region of the dendritic spines and faces the excitatory synaptic terminals *(Luja´n et al., 1996; Luja´n et al., 1997)*. In contrast, mGluR1 is not found on CA1 dendrites *(Shigemoto et al., 1997)*, indicating that mGluR5 is a major receptor at their excitatory synapses which activates the Gq-PLC cascade.

To examine whether the attenuated calcium response to glutamate, NMDA and DHPG could be due to reduction in receptor availability, we next determined the cell surface expression of receptors in control and lithium-treated hippocampal neurons using chemical biotinylation technique. Seven days of lithium treatment significantly reduced the cell surface expression of mGluR5, the predominant group 1 mGluR receptor. There was no significant reduction in the non-biotinylated intracellular fraction of mGluR5. In contrast, seven days of treatment with lithium did not significantly influence the cell surface expression of the NMDA receptor. The non-biotinylated intracellular fraction of NR1 was not detectable in the lanes loaded with the intracellular cell lysate, which is in agreement with the fact that NMDA receptors are membrane receptors.

Several lines of evidence indicate that both mood disorders and schizophrenia are associated with disturbances in the glutamate system. Our results show that lithium has a dual effect on glutamate calcium signaling. Since NMDA receptors are ligand-activated calcium channels and mGluR1/5 R are G protein– coupled receptors (GPCRs) as well as activators of IP3, the mechanisms of action must be different. Our results are in accordance with the finding by Nonaka et al., where lithium attenuated the response of NMDA receptors activation *(Nonaka et al., 1998)*. The attenuation in the NMDA

response was not found to be associated with changes in the receptor expression on the plasma membrane. It has been suggested that lithium may have an affinity to proteins similar to that reported for magnesium *(Mota De Freitas et al., 2006)*. Through our data, we did not find any immediate effect of lithium on the NMDA calcium response *(Bertolino and Vicini, 1988).* This appears to exclude the possibility that lithium would, like magnesium, act by plugging the calcium channel of the NMDA receptor.

We demonstrated that lithium attenuates the response from activated mGluR1/5 receptors, which was not previously known. The decrease in the plasma membrane expression of mGluR5 we found in association with the attenuation of the calcium response of the mGluR1/5 may be due to disruption in the contact with anchoring protein. The mGluR1/5 receptors are known to be linked to several proteins including the Homer protein, and to the postsynaptic density. Although it cannot be proved from the present results, it is thus likely that the decrease of the calcium peak amplitude, following mGluR5 receptors activation, may be partially explained by reduced expression of functional receptors.

It has been reported by *(Pisani et al., 2001)*, that group I mGluRs have a facilitatory role on the response of NMDA receptors via mGluR5 activation. In that study, it was shown that DHPG enhancement of the NMDA receptor response was abolished in mGluR5 knockout mice. It has been also reported that null-mutant mice lacking mGluR5 have a partial impairment in NMDA receptor-dependent LTP *(Lu et al., 1997).*Thus it is possible that the attenuated NMDA effect observed is secondary to the downregulation of mGluR5 membrane expression and the attenuated calcium response following mGluR1/5 receptor activation.

4.2.2 Lithium effect on Gq-coupled receptors

Group 1 mGluRs are Gq-protein-coupled receptors which are coupled to the activation of PLC, generation of IP3 and calcium release from the intracellular stores. Here we have tested if the effect of seven days of lithium treatment is specific for the mGluRs-mediated $Ca2^{+}$ response or whether this effect can also be observed for other receptors coupled to the PLC-IP3 signaling pathway. Carbachol is an agonist of the Gq-coupled muscarinic cholinergic receptors. We found that the carbachol-mediated Ca^{2+} response was significantly attenuated in hippocampal neurons treated seven days with lithium.

Taken together, our results showed that the long-term exposure to lithium attenuated the response of two Gq-coupled receptors; the mGluR1/5 receptors and the

acetylcholine receptor. Several lines of evidence suggest that long term exposure to lithium affects the association between GPCRs and other proteins *(Hahn et al., 2005)*. Lithium has been shown to attenuate cyclic adenosine monophosphate (cAMP) generation triggered by Gs-coupled receptors *(Carli et al.1994)* and has also been reported to interrupt the signaling pathway of the Gi-coupled serotonin receptors *(Wang and Friedman, 1999)*. A recent study from Caron's group *(Beaulieu et al., 2008)* has shown that lithium interrupts the downstream signaling of beta-arrestin 2, a GPCR scaffolding protein.

4.2.3 Lithium effect on Ca^{2+} levels

To record the absolute values for $[Ca^{2+}]_i$ in control and lithium-treated cells, we performed calibration experiments of $[Ca^{2+}]_i$. The basal level of $[Ca^{2+}]_i$ was 116 $\pm$10 nM in control cells, and 87 $\pm$ 11 nM in lithium-treated cells. The difference between the two groups was significant. It is possible that the decreased influx of calcium via the NMDA calcium channel as well as other, above-mentioned, receptor-operated Ca2_ entries, has contributed to the reduction in $[Ca^{2+}]_i$ level.

The sarco(endo)plasmic reticulum Ca^{2+}-ATPase (SERCA) pump, which is responsible of the active uptake of calcium to the ER, can be specifically inhibited by CPA *(Goeger et al., 1988)*. Following inhibition of the SERCA pump, Ca^{2+} will leak out of the ER, resulting in a transient increase of $[Ca^{2+}]_i$ *(Hernández-Fonseca and Massieu, 2005)*. The magnitude of this increase may be used as an index of how much calcium has been stored in the ER. We found that seven days of lithium treatment leads to reduction of the CPA-mediated cytosolic Ca^{2+} increase. This indicates that the ER calcium stores are reduced in cells exposed to seven days of lithium treatment. Both mGluR5 and muscarinic receptors are coupled to G-protein PLC–IP3 Ca^{2+} signaling pathway. Therefore the observed reduction in ER calcium level may have an important role in the attenuation of these receptor-activated Ca^{2+} responses. Store-operated Ca^{2+} channels are activated in response to depletion of the ER store and allow Ca^{2+} to enter the cell and refuel the ER *(Putney, 1999)*. The lithium-induced reduction in ER calcium stores may, due to effect on the store-operated calcium entry, also contribute to the observed alteration in $[Ca^{2+}]_i$ levels.

This study has shown that lithium affects many aspects of $[Ca^{2+}]_i$ levels and turnover. Since Ca2+ is considered to be the most common versatile signaling molecule in neurons, the observed effects can be expected to influence the communication within and between neurons in a variety of ways. Thus it is not surprising that lithium has been

shown to have a variety of functional, pharmacological and developmental effects. However, further studies are required to address questions around the actual effect of lithium on intracellular calcium.

4.3 5-HT1BR; A NOVEL THERAPEUTIC TARGET FOR TREATING DEPRESSION (PAPER III)

Postsynaptic and dedritic receptors' expression, organization and trafficking have become an important target for novel neuropharmacological therapy research. The 5-HT1BR is implicated in mood disorders such as depression. In this study we investigated the localization, organization and trafficking of 5-HT1B receptor.

4.3.1 5-HT1BRs are abundant in intracellular stores

To investigate the expression of native 5-HT1BR in hippocampal neurons dendrites, we performed immunolabeling of primary cells in culture, after membrane permeation. The majority of the native receptor immunoreactivity in dendrites was highly clustered. Image magnification of a dendrite branch clearly showed a strong contrast between clustered and diffuse membrane immunoreactivity. Contrarily, immunolabeling without cell permeation resulted in diffuse labeling with no clusters, indicating that the majority of endogenous 5-HT1BRs in the dendrites are intracellular. It seemed likely that the intracellular clusters represents vesicle containing multiple 5-HT1BRs. Performing subcellular fractionation of adult hippocampal tissue, provided further evidence of a predominant vesicular expression of 5-HT1BR.

To study the appearance of 5-HT1BR in living cells, hippocampus neurons in primary cultures were transfected with fluorescently labeled receptors. As seen with endogenous receptors, the labeled 5-HT1BRs were localized prominently in intracellular aggregates in the dendrites. Live-cell immunolabeling of 5-HT1BRs with a small, extracellular hemagglutinin tag resulted in negligible reactivity clustering in dendrites. Fixation and membrane permeation before immunolabeling resulted in aggregate reactivity in the dendrites, further suggesting that 5-HT1BR aggregates are not found in the plasma membrane and instead are intracellular aggregates.

To compare the expression of 5-HT1BR with other receptos, a number of other neurotransmitter GPCRs, including 5-HT1AR, 5-HT4R, D1R, D2R, D5R, and mGluR5, were also fluorescently labeled and expressed in living cells. None of these GPCRs were expressed extensively in intracellular aggregates. Magnified confocal images of dendrite fragments show a striking contrast between expression of

5-HT1BRs and all other examined GPCRs. Unlike the 5-HT1BR, the 5-HT1AR, 5-HT4R, D1R, D2R and D5R were highly expressed in the dendritic membrane and showed no discernable intracellular aggregates.

These results indicate that, contrary to other GPCRs, 5-HT1BRs has a unique appearance of aggregates in the dendrites of hippocampal neurons. Subcellular fractionation results strengthen the idea that these aggregates are vesicles. Even though vesicles are a prominent component of presynaptic machinery, they are rarely observed post-synaptically. A recent ultrastructural study by *(Peddie et al., 2008)* provided strong evidence of the existence of large postsynaptic vesicles carrying the 5-HT1BR in hippocampus neurons.

4.3.2 5-HT1BRs are mobile in the dendritic membrane and transported in vesicles

Receptor mobility is crucial for regulating receptor distribution in the cell. To analyze the transport of different receptors in the dendrites, we used Fluorescence Recovery After Photobleaching (FRAP) technique. Kymographs of 5-HT1BR FRAP recordings indicate that there are two distinct modes of 5-HT1BR transport: intracellular aggregate trafficking and lateral membrane diffusion. To investigate how unique this internal trafficking of the 5-HT1BR is among GPCRs, we compared dendrite FRAP recordings of the 5-HT1AR, 5-HT4R, D1R, D2R, D5R, and mGluR5. No aggregate movement was detectable in any of these GPCR recordings, rather lateral diffusion movement was seen as fluorescence increase in the bleached fragments. The increase in the fluorescence was consistently slow and diffuse, indicating gradual population recovery in the bleached regions. Moreover, intensity curves of smaller analysis region showed striking intensity changes of 5-HT1BR, which was not observed for other GPCRs, emphasizing the transport of internal aggregates of 5-HT1BRs. A full description of the FRAP technique and the different analysis and interpretations of the kymograph is described in the experimental procedures section.

To visualize the different expression levels in transport vesicles and the plasma membrane, we also used total internal reflection fluorescence microscopy (TIR-FM). TIR-FM limits fluorescence excitation to within 100nm of the culture cover slip surface, thereby isolating attached membrane and intracellular components in proximity to the membrane. From TIR-FM one can detect active transport of 5-HT1BRs in the dendrites that is close to the dendritic membranes. It also

demonstrates the strong contrast between receptor density in transport vesicles and in the plasma membrane.

To further understand the mode of transport of distinct intracellular 5-HT1BRs aggregates, we examined sensitivity to agents that disrupt polymerization of transport filaments. One mode of active transport is via microtubule tracks and is driven by ATP consumption *(Gilbert et al., 1985)*. Disruption of microtubule polymerization with nocodazole fragmented the trafficking of discrete 5-HT1BR aggregates. Actin disruption with latrunculin-A had little effect on 5-HT1BR trafficking. We found also that aggregate transport in dendrites is highly dependent on temperature.

For further characterization of 5-TH1BR aggregates, we searched for parallel expression with a known vesicular protein. When we co-transfected 5-HT1BRs with calcyon, a vesicular protein in the brain *(Lidow et al., 2001, Kruusmägiet al., 2007)*, we identified an overlap of expression in cultured neurons. Many of the prominent vesicles containing calcyon also contained 5-HT1BRs. Time-lapse imaging of the two proteins in neuroblastoma cells also revealed movement of vesicles containing both 5-HT1BRs and calcyon. All the results presented above are consistent with vesicular expression and transport of 5-HT1BRs in dendrites.

4.3.3 5-HT1BR recruitment occurs at the dendritic membrane

Our findings showed that most of the 5-HT1BRs appear to be maintained in intracellular vesicles and that only a small fraction diffuses to the plasma membrane. Functional synapses are the present on dendritic membrane, explaining the dependence of postsynaptic 5-HT1BRs function on the mode by which the receptors stored in the vesicles are delivered to the plasma membrane. To study the process by which the receptor storage vesicles interacts with the plasma membrane and how the vesicular 5-HT1BRs are delivered to the plasma membrane, we expressed 5-HT1BRs labeled with an extracellular pH-sensitive fluorescent protein, superecliptic pHluorin (SpH). Expression of SpH in the vesicle lumen resulted in dimming of the fluorochrome, due to the highly acidic environment. Exocytosis changed the SpH environment from the acidic vesicle lumen to a neutral extracellular environment, thereby enabling fluorescence *(Burrone et al., 2006)* and providing a vesicle fusion event marker. This approach has previously been used to assess the exocytosis of recycled receptors by measuring fluorescence accumulation over long periods of time *(Makino and Malino, 2009, Petrini et al., 2009)*. SpH-labeling has also been used to resolve single vesicle

exocytotic events with line scan analysis to suggest diffusion from the docking site *(Yudowski et al., 2006)*.

To monitor 5-HT1BRs delivery from vesicle stores to the plasma membrane, we performed time-lapse recordings on dendrites of neurons expressing 5-HT1BR-SpH. Exocytosis of vesicles containing 5-HT1BR-SpH was detected as a dramatic increase of confined fluorescence and subsequent loss of intensity. The time course of 5-HT1BR exocytosis was recorded as fluorescence decay after fusion with the plasma membrane. The mean fluorescence profile shows an abrupt increase in fluorescence intensity followed by a rapid decay. Fluorescence loss indicates receptors leaving the fusion site, which can occur either by lateral diffusion or immediate internalization. Surface plots of single exocytotic events show the initially confined fluorescence at the docking site. In later recording frames, the confined receptors appear to spread homogeneously into the surrounding membrane, suggesting lateral diffusion.

To measure the receptors diffusion from the docking site to the surrounding membrane, we demonstrated a two-dimensional lateral spread of receptors from the exocytotic site to the surrounding membrane after delivery of single vesicles. We mapped each 5-HT1BR-SpH exocytotic event with two regions, one defining the initial event area and a surrounding region with twice the inner area. The total integrated intensity profile of the inner region from one exocytotic event demonstrated an abrupt increase in fluorescence. This indicate vesicle docking, immediately followed by an exponential-like decay which then settles at a level slightly above the initial intensity. The intensity profile of the surrounding area also increased during the fusion event, but there is a time difference between intensity peaks of the two regions, due to passive diffusion of 5-HT1BRs from the site of exocytosis. This observation supports the idea that each vesicle carries many copies of 5-HT1BR.

4.3.4 Preferential sites of dendritic 5-HT1BR recruitment

As spatial organization of proteins in the dendrites is a major determinant of cellular function, we explored the idea of preferential exocytosis sites. Recurrent exocytosis is expected at presynaptic terminals *(Gaffield et al., 2009)*, but very little is known about sites of preferential exocytosis in the dendritic membrane. Time-lapse recordings of 5-HT1BR-SpH describe the extent of spatial proximity of 5-HT1BR-SpH exocytosis events. We estimated that 78$\pm$ 8% of recorded exocytotic events (334 total events in 8 cells) in each neuron correspond to regions of repeated exocytosis. From that one can conclude that there are preferential regions of the dendritic plasma membrane that

exhibit repetitive exocytosis of 5-HT1BRs. Since the lipid composition of the plasma membrane is considered to play a role in the efficiency of the exocytotic process *(Lang et al., 2001, Chamberlain et al., 2001)*, it is possible that membrane patches of altered lipid composition, such as lipid rafts, provide inherent sites of increased incorporation. We have observed in ongoing studies that polyunsaturated fatty acid treatment results in increased 5-HT1BR expression in the membrane, possibly as a result of increasing preferential membrane recruitment sites.

4.3.5 Gephyrin interaction with the 5-HT1BR

To identify a potential cofactor that determines the preferential sites of exocytotic activity, we investigated the interaction between 5-HT1BRs and synaptic scaffold proteins. Through co-immunoprecipitation experiments using tissue homogenate from adult rat hippocampus, we detected a strong interaction between 5-HT1BRs and gephyrin, the principle scaffold molecule at inhibitory synapses. We found however no interaction between 5-HT1BRs and PSD-95 or Homer, which are recognized as principle neuronal excitatory scaffold proteins.

If 5-HT1BRs are recruited at inhibitory synapses, it is probable that the exocytotic event would occur at or near gephyrin clusters. To determine if gephyrin present a preferential site for 5-HT1BR exocytosis, we expressed fluorescent gephyrin together with 5HT1bR-SpH. We found that 44 $\pm$ 6% of 5-HT1BR -SpH preferential recruitment sites overlap with gephyrin clusters, suggesting a possible role for these clusters in recruitment of 5-HT1BRs to the dendritic membrane. On the other hand, gephyrin clusters have been reported to be mobile *(Hanus et al., 2006)* and localized at both synaptic and non-synaptic clusters *(Danglot et al., 2003, Calamai et al., 2009)*, therefore the co-localization of preferential exocytosis sites with gephyrin clusters or inhibitory synapses is only an estimate.

The association of dendritic 5-HT1BRs with inhibitory synapses is consistent with the concept that presynaptic 5-HT1BRs play an inhibitory role in axons. However, the insertion of 5-HT1BRs into the plasma membrane does not preferentially take place at the site of inhibitory synapses. Conversly, results reported on the AMPAR revealed the exocytosis of the receptor at or in proximity to excitatory synapses *(Makino and Malino, 2009, Petrini et al., 2009)*. This feature points to an interesting difference between exocytosis of the locally recycled AMPARs and that of 5-HT1BRs, highlighting the concept of delivering multiple copies of the receptors to the plasma membrane in an activity-dependent manner.

4.3.6 Dendritic 5-HT1BR recruitment is associated with cellular activity

To further examine the effect of cellular activity on receptor transport, we studied the consequence of neural activity elevation on receptor recruitment. We abruptly increased the extracellular concentration K^+ to elevate the neuronal activity in the cultured neurons, thereby depolarizing the neuronal membrane. K^+ stimulation generated a temporal increase in cell firing rate, which was associated with significant increase in 5-HT1BR-SpH recruitment to the plasma membrane. The receptor recruitment in response to neuronal activity together with the previous findings of 5-HT1BRs association with inhibitory synapses may entail that such recruitment can serve to dampen excessive neuronal activity.

It is generally accepted that presynaptic vesicles transport is energy-demanding. The fact that post synaptic 5-HT1BR vesicle transport was highly temperature dependent and their arrest after microtubules disruption, both indicate that they are also driven by energy demanding process. The lateral diffusion movement of membrane proteins is an energy-efficient process following the laws of Brownian movement and is the principle means of receptor transport. The emergence of energy-demanding intracellular transport of 5-HT1BRs suggests that uncontrolled postsynaptic membrane expression of this receptor could be harmful to neuronal function. Therefore the maintenance of 5-HT1BRs stored in intracellular vesicles may be a necessary mechanism to prevent not unnecessary receptors from diffusing freely into the synapses.

5 CONCLUSIONS AND FUTURE PERSPECTIVES

5.1 MHC-I, POSSIBLE INVOLVEMENT IN THE PATHOGENESIS OF MOOD DISORDERS

The differential levels of brain development at various ages may be responsible for the differences observed in the clinical manifestations and the response to therapy between children and adults. This study highlights the fact that the expression of MHC-I and CD3ζ in the hippocampus is vulnerable during selective periods of development. Speculations of a link between neuropsychiatric disorders and environmental effects such as brain insults occurring early in life, has been previously made. Our study emphasized the role of TNF-α as a factor that could alter the MHC-I/CD3ζ signaling system. Taking into account the dual role of MHC-I and CD3ζ molecules as regulators of development and plasticity in the CNS, alterations in their expression levels may be involved in the pathogenesis of neuropsychiatric disorders. Further research techniques, knockout mice, behavioral studies and antibodies that can recognize individual MHC-I proteins are required to get a deeper insight on the possible role of this signaling system in the pathogenesis of neuropsychiatric disorders.

5.2 CALCIUM SIGNALING, POTENTIAL TARGET FOR BIPOLAR DISORDER TREATMENT

Understanding the mechanism of drug action can aid in understanding disease pathology and help develop more target-specific medications. In our study we highlight the down-regulation of calcium as a potential target for lithium action. Together with previous reports on the hyperactivity of intracellular calcium ion mobilization in the peripheral cells of bipolar patients, one can speculate that calcium hyperactivity may play a role in the pathogenesis of bipolar disorder. Further research is required to study calcium homeostasis in the brain of bipolar patients, and to develop more targeted medications with fewer side effects.

Calcium homeostasis is crucial for normal-functioning neuronal circuits and for the synaptic plasticity of the brain. Furthermore, this is fundamental for brain function in adulthood, more specifically during development. This indicates that the use of lithium during childhood and adolescence is suitable, where neuronal circuits and synapses are in the phase of maturation. Moreover, the long term effect of lithium on less mature brain should be considered. Although rat animal studies shows that

chronic lithium treatment magnifies learning in rats, more clinical research is required to follow the learning and cognition abilities of children and adolescents treated with lithium.

5.3 5-HT1BRS, POTENTIAL TARGET FOR ANTI-DEPRESSIVE THERAPY

Most research nowadays on the effects of neuropharmacological therapy is directed towards studying dendritic receptor expression and organization as main target in pathology and treatment of neuropsychiatric disorders. 5-HT system dysfunction is implicated in mood disorders especially depression, where SSRIs are widely used as anti-depressive therapy. Although SSRIs are highly efficient therapies, their use among children and adolescents is still controversial due to their severe side effects. A probable cause of such side-effects is the indiscriminate activation of all 5-HT receptors at the synapse. Targeting therapy towards the regulation of post synaptic receptor subtypes may enable more controlled and directed modulation of 5-HT signaling; thereby increasing the treatment specificity. 5-HT1B, 5-HT1B and 5-HT4 are among the 5-HT receptors implicated in mood disorders. The unique distribution and vesicle trafficking we described in our study, sets 5-HT1BRs apart from the majority of postsynaptic receptors and opens a new channel for a receptor-specific approach to 5-HT signal regulation. Further *in vivo* and *in vitro* studies on the effect of polyunsaturated fatty acids on the expression and function of 5-HT1BRs could provide a new line of therapy for depressive disorders.

6 ACKNOWLEDGEMENTS

Writing this thesis is one of the big steps in my life that provided the opportunity to stop, look backward and evaluate how much I learned in science and how much I developed as a person. At this stage, I would like to thank my *God* for all His kindness, for the life He gave me and for all the opportunities and people He allowed me to meet. Every person I met in my life drew a line in my personality and each scientist I met since I entered to the world of research has influenced the development of this thesis in a way or another. However, a certain group of people had a stronger imprint by their knowledge, help and support. Therefore, I would like to take the opportunity to express my deep gratitude to those who contributed most to this work; hope words would help to formulate that to:

My main Supervisor professor *Anita Aperia*; for accepting me in her group, teaching and inspiring me to be a scientist, encouraging me to work as a clinician even guiding in choosing my future speciality and mostly for her patience and understanding during the periods of frustration during writing this thesis. Thanks for having always the time to listen and advice me, and for the great effort in developing this thesis.

Professor *Per-Anders Rydelius*; for his patience, kindness, encouragement and endless support. Thanks for always having time for fruitful discussions that always answered my questions, for enthusiastically encouraging me to continue in the clinical career and mostly for reading and revising this thesis word by word with constructive valuable comments.

Senior Scientist *Sergey Zelenin*; for his teaching on how to be an organized thorough researcher that knows how to control and follow the experiments.

My co-writers and friends; *Ann-Christine Eklöf*, for sharing her massive experience about animal studies and science as well as her wise experience in life that made me try to be a stronger woman capable of dealing with life situations logically more than emotionally. *Oleg Aizman*, for sharing his expertise in calcium signaling and for being an ideal guide towards the clinical career. *Lena Scott*, my special fellow, for always having the time for discussions, for being the honest listener and for giving nice replies constantly. *Thomas Liebmann*, it has been pleasant to work together, thanks for extensive teaching about imaging and constructs, for being patient with all the questions and discussions and mostly for the great support during the preparation for this thesis. *Markus Kruusmägi*, room-mate and computer expert, thanks for the rapid, unselfish, always available and efficient help and support when it comes to computers,

for being the first to teach me about microscopes and imaging and for the patience with all the questions. *Alexander Bondar*, thanks for the experienced extensive answers to the molecular biology questions. *Hjalmar Brismar*, thanks for adequate answers to many technical questions. *Paul Greengard*, *Marc Flajolet* and *Per Svenningsson* for fruitful collaboration.

Raija Wallenborg; for excellent secretarial work, for nice morning chats, for being always a good understanding listener and mostly for the extensive help and support during the preparation for defending this thesis.

Gunilla Nilsson; thanks for the welcoming smile and for the help when needed.

My room-mates; *Eli Gunnarson*, my sincere admirable friend and supporter, thanks for the nice chats, honest advices, open heart and for keeping my sanity by sharing life situations. *Linda Westin*, for the pleasant company.

My co-workers; *Lill-Britt Svensson*, for always helping me even with last minute orders, for nice chats every now and then and for expert immunohistochemistry work, even if it was not included in the thesis, thanks for the great experience I gained. *Eivor Markus*; for sharing her experience in all types of cultures, mostly primary hippocampus cultures, and for so many pleasant chats. *Louise Gustafsson*, for support and help with cell culture work and for sharing life experiences. *Mona Agren*, the western blot lady, for providing me with the fundamental basics to run a successful western blot. *Camella Hammar* and *Josefin Forsberg*, for great help and support during running animal experiments, and for always finding extra rat heads.

Present and former colleagues; *Marina Zelenina*, for being the first to teach me western blot and for always having time for many questions. *Jerry DiBona*, for having the time to share and discuss his medical and scientific experience. *Susanne Crambert*, for the fruitful discussions about the biotinylation and the cAMP. *Xiaoli Liu*, for being helpful and for pleasant coffee times. *Ulla Holtbäck*, for being always willing to help. *Siobhan Connor*, for the nice chats and for her thorough linguistic revision of essential parts of this thesis. *Georgiy Khodus*, for answering all the statistics questions that supported us in the lithium paper. *Nina Illarionova*, *Zuzana Spicarova*, *Yutong Song*, *Evgeniya Burlaka*, *Jacopo Fontana*, *Carolina Rigos* and *Rachel Vieux*, for creating inspiring scientific working atmosphere and for always willing to help in many ways. *Rochellys Diaz Heijtz, Ayako Miyakawa, Mark Lal, Per uhlen, Åsa Laestadius, Maria Sol Kruse, Juan Li* and *Yanhong Li*, for being excellent models and bright lights in the road of research in our laboratory.

All the families and friends from the Coptic Orthodox Church in Sweden and Egypt, thanks for all the prayers, support and care. Special Thanks goes to *Dr. Soheir Beshara*, the ideal model, for being my dear wise secret mentor and for all her help, support and prayers.

My dear parents, *Onsi Sourial* and *Soheir Girgis*, for providing me with the essentials for a successful prosperous life, for their teaching about the real meaning of being focused, organized and independent, for their supporting in all my decisions and for offering their best help to when needed.

My tender warmhearted sister, *Sister Ruth,* for her care, prayers, concern and support, despite the distance.

My beloved caring brother, *Samer Sourial*, for his unselfish help and support, for the wise scientific advices, for the nice pleasant chats and especially for the patient thorough revision and correction of this thesis.

My affectionate lovesome husband, *Gamal Bassilious*, my life companion, for sharing and supporting me in every step, for wonderful moments in an amazing life with adorable kids, for the unselfish help and understanding during the tough incidences and for his honest hard work to offer the best.

My precious jewels, priceless gifts, true meaning of life and adorable children; My first happiness and supporter, *George*, for being there in every step, following the progress of the thesis preparation and for the excellent help with the writing. My special unique *Mary*, for being the second mother when I am absent, for sharing me the responsibility and for hundreds cups of coffee during writing this thesis. My hearts' joy, *Joy*, for the secret special moments, for sharing me my thoughts and for her sensitive, understanding and lovely discussions. My heavenly present, *Janie* and *John*, their smiles, love, hugs and kisses are my real nourishment and support. Thank you all for your considerate understanding in the moments when I could not be a good mother.

My cherished spiritual father, *Bishop Barsoum*, words would stand disabled when trying to describe my gratitude to his extraordinary care and support and the exceptional wise guidance in all my life steps.

The work in this thesis was supported by grants from the Swedish Research Council, the Märtha and Gunnar V. Philipson Foundation and the Persson Family Foundation.

"It always seems impossible until its done." Nelson Mandela

7 REFERENCES

- Abe T, Sugihara H, Nawa H, Shigemoto R, Mizuno N and Nakanishi S (1992). Molecular characterization of a novel metabotropic glutamate receptor mGluR5 coupled to inositol phosphate/Ca 2_ signal transduction. J Biol Chem 267:13361–13368.
- Abou-Saleh MT (2006). Neuroimaging in psychiatry: An update. J Psychosom Res; 61: 289–293
- Adler CM, DelBello MP, Jarvis K, Levine A, Adams J and Strakowski SM (2007). Voxel-based study of structural changes in first-episode patients with bipolar disorder, Biol. Psychiatry 61, pp. 776–781.
- Aly H, Khashaba MT, El-Ayouty M, El-Sayed O and Hasanein BM (2006) IL-1_, IL-6 and TNF-_ and outcomes of neonatal hypoxic ischemic encephalopathy. Brain Dev 28:178–182
- Amaral DG (1978). A golgi study of cell types in the hilar region of the hippocampus in the rat. Journal of Comparative Neurology, 182: 851-914.
- Amaral DG and Witter MP (1989). The three-dimensional organization of the hippocampal formation: a review of anatomical data. Neuroscience, 31: 571-591.
- Amaral DG and Insausti R (1990). Hippocampal formation. In: Praxinos G, editor. The human nervous system. San Diego: Academic Press. p 711–755.
- Amaral, D and Lavenex P (2006). "Ch 3. Hippocampal Neuroanatomy". in Andersen P, Morris R, Amaral D, Bliss T, and O'Keefe J. The Hippocampus Book. Oxford University Press.
- Andersen P (1975). Organization of hippocampal neurons and their interconnections. In R.L. Isaacson & K.H. Pribram (Eds.) The Hippocampus Vol. I (pp. 155-175), New York, Plenum Press.
- Anforth HR, Bluthe RM, Bristow A, Hopkins S, Lenczowski MJ, Luheshi G, Lundkvist J, Michaud B, Mistry Y, Van Dam AM, Zhen C, Dantzer R, Poole S, Rothwell NJ, Tilders FJ and Wollman EE 1998 Biological activity and brain actions of recombinant rat interleukin-1alpha and interleukin-1beta. Eur Cytokine Netw 9:279– 288
- Arnold SE and Trojanowski JQ (1996): Human fetal hippocampal development. 1. Cytoarchitecture, myeloarchitecture, and neuronal morphologic features. J Comp Neurol 367: 274– 292.
- Ashby MC, Maier SR, Nishimune A and Henley JM (2006). Lateral diffusion drives constitutive exchange of AMPA receptors at dendritic spines and is regulated by spine morphology. J Neurosci; 26(26):7046-55.
- Bachevalier J, Beauregard M and Alvarado M (1999).Long-term effect of neonatal damage to the hippocampal formation and amygdaloid cortex on object discrimination learning and object recognition memory in monkeys, Behav Neurosci 113, pp. 1127–1151.
- Bakker ABH, Wu J, Phillips JH and Lanier LL (2000): NK cell activation: distinct stimulatory pathways counterbalancing inhibitory signals. Hum Immunol, 61:18-27.
- Balu DT and Lucki I (2009). Adult hippocampal neurogenesis: regulation, functional implications, and contribution to disease pathology. Neurosci Biobehav Rev; 33(3):232-52.
- Banker G and Cowan W (1977). Rat hippocampal neurons in dispersed cell culture. Brain Res 126:397–425
- Banker G and Cowan W (1979). Further observations on hippocampal neurons in dispersed cell cultures. J Comp Neurol 187:469–494

- Banker G and Waxman A (1988). Hippocampal neurons generate natural shapes in cell culture. In: Lasek R, Black MB (eds) Intrinsic determinants of neuronal form. Liss, New York
- Bartlett WP and Banker G (1984). An electron microscopic study of the development of axons and dendrites by hippocampal neurons in culture. II Synaptic relationships. J Neurosci 4:1954–1965
- Bartha AI, Foster-Barber A, Miller SP, Vigneron DB, Glidden DV, Barkovich AJ and Ferriero DM (2004). Neonatal encephalopathy: association of cytokines with MR spectroscopy and outcome. Pediatr Res 56:960–966
- Bauer M, Alda M, Priller J and Young LT (2003). International Group for the Study of Lithium Treated Patients (IGSLI). Implications of the neuroprotective effects of lithium for the treatment of bipolar and neurodegenerative disorders. Pharmacopsychiatry 36 (Suppl 3):S250–S254.
- Bayer S (1985). Hippocampal region. In G. Paxinos (Ed.) The Rat Nervous System Vol1; Forebrain and Midbrain (pp. 335-352), New York, Academic Press.
- Bear, Mark F, Connors, Barry W, Paradiso *and* Michael A. Neuroscience*:* exploring the brain. 2ed. Chapter 7, page 163-252.
- Beattie EC, Stellwagen D, Morishita W, Bresnahan JC, Ha BK, Von Zastrow M, Beattie MS and Malenka RC (2002). Control of synaptic strength by glial TNFalpha. Science 295, 2282–2285.
- Beaulieu JM, Marion S, Rodriguiz RM, Medvedev IO, Sotnikova TD, Ghisi V, Wetsel WC, Lefkowitz RJ, Gainetdinov RR and Caron MG (2008) A beta-arrestin 2 signaling complex mediates lithium action on behavior. Cell 132(1):125–136.
- Behr J, Wozny C, Fidzinski P and Schmitz D (2009). Synaptic plasticity in the subiculum. Prog Neurobiol. 89(4):334-42.
- Belet N, Belet U, Incesu L, Uysal S, Ozinal S, Keskin T, Sunter AT and Kucukoduk S (2004). Hypoxic-ischemic encephalopathy: correlation of serial MRI and outcome. Pediatr Neurol 31:267–274
- Benedetti F, Barbini B, Bernasconi A, Fulgosi MC, Colombo C, Dallaspezia S, Gavinelli C, Marino E, Pirovano A, Radaelli D and Smeraldi E (2008). Serotonin 5-HT2A receptor gene variants influence antidepressant response to repeated total sleep deprivation in bipolar depression. Prog Neuropsychopharmacol Biol Psychiatry;32 (8):1863-6.
- Benes FM, Turtle M, Khan Y and Farol P (1994). Myelination of a key relay zone in the hippocampal formation occurs in the human brain during childhood, adolescence, and adulthood, Arch Gen Psychiatry 51, pp. 477–484.
- Benes FM, Todtenkopf MS and Kostoulakos P (2001). GluR567 subunit immunoreactivity on apical pyramidal cell dendrites in hippocampus of schizophrenics and manic depressives, Hippocampus 11**,** pp. 482–491.
- Beneyto M, Kristiansen LV, Oni-Orisan A, McCullumsmith RE and Meador-Woodruff JH (2007). Abnormal glutamate receptor expression in the medial temporal lobe in schizophrenia and mood disorders, Neuropsychopharmacology 32, pp. 1888–1902
- Bennett MR (2010). Synapse regression in depression: the role of 5-HT receptors in modulating NMDA receptor function and synaptic plasticity. Aust N Z J Psychiatry. Apr;44(4):301-8.
- Berridge MJ, Downes CP and Hanley MR (1989). Neural and developmental actions of lithium: a unifying hypothesis. Cell 59:411–419.

- Bertolino M and Vicini S (1988). Voltage-dependent block by strychnine of N-methyl-D-aspartic acid-activated cationic channels in rat cortical neurons in culture. Mol Pharmacol 34(2):98–103.
- Besnier N, Fakra E, Kaladjian A, Adida M, Maurel M and Azorin JM (2010). Depressive onset episode of bipolar disorder: clinical and prognostic considerations. Encephale.;36 Suppl 1:S18-22.
- Birmaher, B., Brent, D., Bernet, W., et al. AACAP Work Group on Quality Issues. (2007). Practice parameter for the assessment and treatment of children and adolescents with depressive disorders. Journal of the American Academy of Child and Adolescent Psychiatry, 46, 503−526.
- Blier P, de Montigny C and Chaput Y (1990). A role for the serotonin system in the mechanism of action of antidepressant treatments: peclinical evidence. J Clin Psychiatry;5:14–20
- Blier P, de Montigny C (1994). Current advances and trends in the treatment of depression.Trends Pharmacol Sci;15:220–226.
- Bliss TVP and Collingridge GL (1993). A synaptic model of memory: long-term potentiation in the hippocampus, Nature 361, pp. 31–39.
- Blumberg HP, Kaufman J, Martin A, Whiteman R, Zhang JH, Gore JC, Charney DS, Krystal JH and Peterson BS (2003). Amygdala and hippocampal volumes in adolescents and adults with bipolar disorder, Arch. Gen. Psychiatry 60, pp. 1201–1208.
- Brunet G, Cerlich B, Robert P, Dumas S, Souetre E and Darcourt G (1990). Open trial of a calcium antagonist, nimodipine, in acute mania. Clin Neuropharmacol 3:224–228.
- Burrone J, Li Z and Murthy VN (2006). Studying vesicle cycling in presynaptic terminals using the genetically encoded probe synaptopHluorin. Nat Protoc.;1(6):2970-8.
- Cai Z, Xiao F, Lee B, Paul IA, and Rhodes PG (1999). Prenatal hypoxia-ischemia alters expression and activity of nitric oxide synthase in the young rat brain and causes learning deficits. Brain Res Bull 49:359–365
- Calamai M, Specht CG, Heller J, Alcor D, Machado P, Vannier C and Triller A (2009). Gephyrin oligomerization controls GlyR mobility and synaptic clustering. J Neurosci. 2009 Jun 17;29(24):7639-48.
- Campbell M, Silva RR, Kafantaris V, Locascio JJ, Gonzalez NM, Lee D and Lynch NS (1991). Predictors of side effects associated with lithium administration in children. Psychopharmacol Bull.;27(3):373-80.
- Carli M, Anand-Srivastava MB, Molina-Holgado E, Dewar KM and Reader TA (1994). Effects of chronic lithium treatments on central dopaminergic receptor systems: G proteins as possible targets. Neurochem Int 24(1):13–22.
- Carroll RC and Zukin RS (2002). NMDA-receptor trafficking and targeting: implications for synaptic transmission and plasticity. Trends Neurosci.;25(11):571-7.
- Catterall WA and Few AP (2008). Calcium channel regulation and presynaptic plasticity. Neuron 59, 882–901.
- Chamberlain LH, Burgoyne RD and Gould GW (2001). SNARE proteins are highly enriched in lipid rafts in PC12 cells: implications for the spatial control of exocytosis. Proc Natl Acad Sci U S A.;98(10):5619-24.
- Chang KD (2010). Course and impact of bipolar disorder in young patients. J Clin Psychiatry. Feb;71(2):e05.
- Cheung AH, Zuckerbrot RA, Jensen PS, Stein REK, Laraque D, and the GLAD PC Steering Committee (2008). Expert survey for the management of adolescent depression in primary care. Pediatrics, 121, 101−107.

- Chew LJ, Takanohashi A, Bell M (2006). Microglia and inflammation: impact on developmental brain injuries. Ment Retard Dev Disabil Res Rev.;12(2):105-12.
- Choquet D and Triller A (2003). The role of receptor diffusion in the organization of the postsynaptic membrane. Nat Rev Neurosci;4(4):251-65.
- Clinton SM and Meadow-Woodruff JH (2004). Abnormalities of the NMDA receptor and associated intracellular molecules in the thalamus in schizophrenia and bipolar disorder, Neuropsychopharmacology 29, pp. 1353–1362.
- Cohen D (2007). Should the use of selective serotonin reuptake inhibitors in child and adolescent depression be banned? Psychother Psychosom.;76(1):5-14.
- Cognet L, Groc L, Lounis B and Choquet D (2006). Multiple routes for glutamate receptor trafficking: surface diffusion and membrane traffic cooperate to bring receptors to synapses. Sci STKE;(327):pe13.
- Coppen A and Wood K (1982). 5-Hydroxytryptamine in the pathogenesis of affective disorders. Adv Biochem Psychopharmacol.;34:249-58.
- Corriveau RA, Huh GS and Shatz CJ (1998). Regulation of class I MHC gene expression in the developing and mature CNS by neural activity. Neuron 21:505–520
- Crair MC and Malenka RC (1995). A critical period for long-termpotentiation at thalamocortical synapses. Nature *375*, 325–328.
- Dammann O, Hagberg H and Leviton A (2001). Is periventricular leukomalacia an axonopathy as well as an oligopathy?, Pediatr. Res. 49, pp. 453–457.
- Danglot L, Triller A and Bessis A (2003). Association of gephyrin with synaptic and extrasynaptic GABAA receptors varies during development in cultured hippocampal neurons. Mol Cell Neurosci.;23(2):264-78.
- De Jong GI, Farkas E, Stienstra CM, Plass JR, Keijser JN, de la Torre JC and Luiten PG (1999). Cerebral hypoperfusion yields capillary damage in the hippocampal CA1 area that correlates with spatial memory impairment. Neuroscience 91:203–210
- Dolan RJ, Poynton AM, Bridges PK and Trimble MR (1990). Altered magnetic resonance white-matter T1 values in patients with affective disorder, Br. J. Psychiatry 157, pp. 107–110.
- Dotti CG, Sullivan CA and Banker G (1988). The establishment of polarity by hippocampal neurons in culture. J Neurosci 8:1454– 1468
- Drevets WC, Price JL and Furey ML (2008). Brain structural and functional abnormalities in mood disorders: implications for neurocircuitry models of depression. Brain Struct Funct.;213(1-2):93-118.
- Dubin MW, Stark LA, and Archer SM (1986). A role for action-potential activity in the development of neuronal connections in the kitten retinogeniculate pathway. J. Neurosci. *6*, 1021–1036.
- Dubovsky SL and Franhs RD (1983). Intracellular calcium ions in affective disorders: a review and a hypothesis. Biol. psychat. 18, 781-797
- Dubovsky SL, Murphy J, Christiano J and lee C (1992). Calcium second messenger system in bipolar disorders data supporting new research directions. J neuropsychiat clin neurosci; 4: 3-14
- Duvernoy HM (2005). The Human Hippocampus: Functional Anatomy, Vascularization and Serial Sections with MRI, 3rd edition . Berlin: Springer-Verlag.
- Eastwood SL and Harrison PJ (2000). Hippocampal synaptic pathology in schizophrenia bipolar disorder and major depression: a study of complexin mRNAs. Mol Psychiatry 5: 425–432.

- Eckenhoff MF and Rakic P (1988): Nature and fate of proliferative cells in the hippocampal dentate gyrus during the life span of the rhesus monkey. J Neurosci 8: 2729–2747.
- Eckenhoff MF and Rakic P (1991): A quantitative analysis of synaptogenesis in the molecular layer of the dentate gyrus in the rhesus monkey. Brain Res Dev Brain Res 64: 129–136.
- Eker C and Gonul AS. Volumetric MRI studies of the hippocampus in major depressive disorder: Meanings of inconsistency and directions for future research. World J Biol Psychiatry 2009; 6: 1–17
- Emslie GJ, Weinberg WA, Rush AJ, Adams RM, and Rintelmann JW (1990). Depressive symptoms by self-report in adolescence: Phase I of the development of a questionnaire for depression by self-report. Journal of Child Neurology, 5, 114–121.
- Feldman DE, Nicoll RA, Malenka RC (1999). Synaptic plasticity at thalamocortical synapses in developing rat somatosensory cortex: LTP, LTD, and silent synapses. J Neurobiol.;41(1):92-101.
- Filley CM (2005). White matter and behavioral neurology, Ann. NY Acad. Sci. 1064, pp. 162–183.
- Findling RL (2009). Diagnosis and treatment of bipolar disorder in young patients. J Clin Psychiatry.;70(12):e45.
- Fletcher TL, De Camilli P and Banker G (1994). Synaptogenesis in hippocampal cultures: evidence indicating that axons and dendrites become competent to form synapses at different stages of neuronal development. J Neurosci 14:6695–6706
- Foland LC, Altshuler LL, Sugar CA, Lee AD, Leow AD, Townsend J, Narr KL, Asuncion DM, Toga AW and Thompson PM (2008). Increased volume of the amygdala and hippocampus in bipolar patients treated with lithium, Neuroreport 19, pp. 221–224.
- Frazer A and Hensler JG (1999). "Understanding the neuroanatomical organization of serotonergic cells in the brain provides insight into the functions of this neurotransmitter". in Siegel G J. Basic Neurochemistry.
- Frey BN, Andreazza AC, Nery FG, Martins MR, Quevedo J, Soares JC and Kapczinski F (2007). The role of hippocampus in the pathophysiology of bipolar disorder. Behav Pharmacol;18(5-6):419-30.
- Friedman DP, Aggleton JP and Saunders RC (2002). Comparison of hippocampal, amygdala, and perirhinal projections to the nucleus accumbens: Combined anterograde and retrograde tracing study in the macaque brain, J. Comp. Neurol. 450, pp. 345–365.
- Frodl T, Möller HJ and Meisenzahl E (2008). Neuroimaging genetics: new perspectives in research on major depression? Acta Psychiatr Scand 118:363–372
- Frodl TS, Koutsouleris N, Bottlender R, Born C, Jager M, Scupin I, Reiser M, Moller HJ and Meisenzahl EM (2008). Depression-related variation in brain morphology over 3 years: effects of stress? Arch Gen Psychiatry 65:1156–1165
- Foster-Barber A, Dickens B and Ferriero DM (2001). Human perinatal asphyxia: correlation of neonatal cytokines with MRI and outcome. Dev Neurosci 23:213–218
- Fumagalli F, Frasca A, Racagni G and Riva MA (2008). Dynamic regulation of glutamatergic postsynaptic activity in rat prefrontal cortex by repeated administration of antipsychotic drugs. Mol Pharmacol.;73(5):1484-90.
- Gaffield MA, Tabares L and Betz WJ (2009). Preferred sites of exocytosis and endocytosis colocalize during high- but not lower-frequency stimulation in mouse motor nerve terminals. J Neurosci.;29(48):15308-16.

- Gerrow K, Romorini S, Nabi SM, Colicos MA, Sala C and El-Husseini A (2006). A preformed complex of postsynaptic proteins is involved in excitatory synapse development. Neuron 49:457–462
- Giedd JN, Vaituzis AC, Hamburger SD, Lange N, Rajapakse JC, Kaysen D, Vauss YC and Rapoport JL (1996): Quantitative MRI of the temporal lobe, amygdala, and hippocampus in normal human development: ages 4–18 years. J Comp Neurol 366: 223–230.
- Gilbert SP, Allen RD and Sloboda RD (1985). Translocation of vesicles from squid axoplasm on flagellar microtubules. Nature.;315(6016):245-8.
- Gleichmann M and Mattson M (2010). Neuronal calcium homeostasis and dysregulation. Antioxid Redox Signal. 2010 Jul 14. [Epub ahead of print]
- Goddard CA, Butts D and Shatz CJ (2007). Regulation of CNS synapses by neuronal MHC Class I, *Proc. Natl. Acad. Sci. USA* 104**,** pp. 6828–6833.
- Goeger DE, Riley RT, Dorner JW and Cole RJ (1988). Cyclopiazonic acid inhibition of the Ca2_-transport ATPase in rat skeletal muscle sarcoplasmic reticulum vesicles. Biochem Pharmacol 37(5):978–981.
- Golden RN, Ekstrom D, Brown TM, Ruegg R, Evans DL, Haggerty JJ Jr, Garbutt JC, Pedersen CA, Mason GA, Browne J, et al (1992). Neuroendocrine effects of intravenous clomipramine in depressed patients and healthy subjects. Am J Psychiatry.;149(9):1168-75.
- Goodman CS and Shatz CJ (1993).Developmental mechanisms that generate precise patterns of neuronal connectivity. Cell.;72 Suppl:77-98.
- Graham DI, Lantos PL, eds (2002). Greenfield's neuropathology, 7thed. London, England Arnold.
- Grant SG, O'Dell TJ, Karl KA, Stein PL, Soriano P and Kandel ER (1992). Impaired long-term potentiation, spatial learning, and hippocampal development in fyn mutant mice. Science;258(5090):1903-10.
- Greer PL and Greenberg ME (2008). From synapse to nucleus: calcium-dependent gene transcription in the control of synapse development and function. Neuron.;59(6):846-60.
- Gruart A and Delgado-García JM (2007). Activity-dependent changes of the hippocampal CA3-CA1 synapse during the acquisition of associative learning in conscious mice. Genes Brain Behav. Suppl 1:24-31.
- Haddjeri N, Blier P and de Montigny C (1998). Long-term antidepressant treatments result in a tonic activation of forebrain 5-HT1A receptors. *J Neurosci*;18:10150–10156.
- Hagino OR, Weller EB, Weller RA, Washing D, Fristad MA and Kontras SB (1995). Untoward effects of lithium treatment in children aged four through six years. J Am Acad Child Adolesc Psychiatry.;34 (12):1584-90.
- Hahn CG, Umapathy, Wang HY, Koneru R, Levinson DF and Friedman E (2005). Lithium and valproic acid treatments reduce PKC activation and receptor-G protein coupling in platelets of bipolar manic patients. J Psychiatr Res 39(4):355–363.
- Hamrin V and Scahill L (2005). Selective serotonin reuptake inhibitors for children and adolescents with major depression: current controversies and recommendations. Issues Ment Health Nurs.;26(4):433-50.
- Hamrin V and Iennaco JD (2010). Psychopharmacology of pediatric bipolar disorder. Expert Rev Neurother.;10(7):1053-88.
- Hanus C, Ehrensperger MV and Triller A(2006). Activity-dependent movements of postsynaptic scaffolds at inhibitory synapses. J Neurosci.;26(17):4586-95.
- Harro J and Oreland L (1996). Depression as a spreading neuronal adjustment disorder. Eur Neuropsychopharmacol;6:207–223.

- Heine M, Groc L, Frischknecht R, Béïque JC, Lounis B, Rumbaugh G, Huganir RL, Cognet L and Choquet D (2008). Surface mobility of postsynaptic AMPARs tunes synaptic transmission. Science;320(5873):201-5.
- Heninger GR, Charney DS and Sternberg DE (1984). Serotonergic function in depression. Prolactin response to intravenous tryptophan in depressed patients and healthy subjects. Arch Gen Psychiatry;41:398–402
- Hernández-Fonseca K and Massieu L (2005). Disruption of endoplasmic reticulum calcium stores is involved in neuronal death induced by glycolysis inhibition in cultured hippocampal neurons. J Neurosci Res 15(2):196–205.
- Higley MJ and Sabatini BL (2008). Calcium signaling in dendrites and spines: practical and functional considerations. Neuron 59, 902–913.
- Houamed KM, Kuijper JL, Gilbert TL, Haldeman BA, O'Hara PJ, Mulvihill ER, Almers W and Hagen FS (1991). Cloning, expression, and gene structure of a G protein-coupled glutamate receptor from rat brain. Science;252(5010):1318-21.
- Hough CJ, Irwin RP, Gao XM, Rogawski MA and Chuang DM (1996). Carbamazepine inhibition of N-methyl-D-aspartate-evoked calcium influx in rat cerebellar granule cells. J Pharmacol Exp Ther 276(1):143–149.
- Howard J and Thompson I (2002). First class way to develop a brain. Nature. 1998 Nov 19;396(6708):219-21.
- Huh GS, Boulanger LM, Du H, Riquelme PA, Brotz TM and Shatz CJ (2000). Functional requirement for class I MHC in CNS development and plasticity. Science.;290(5499):2155-9.
- Humphrey T (1967): The development of the human hippocampal fissure. J Anat; 101: 655– 676.
- Ingram R, and Trenary L (2005). Mood disorders. In J. E. Maddux, & B. A. Winstead (Eds.), Psychopathology: Foundations for a contemporary understanding (pp. 155–177). New Jersey: Lawrence Erlbaum Associates.
- Isomaki P, Panesar M, Annenkov A, Clark JM, Foxwell BM, Chernajovsky Y and Cope AP (2001). Prolonged exposure of T-cells to TNF down-regulates TCR zeta and expression of the TCR/CD3 complex at the cell surface. J Immunol 166:5495–5507
- Jacob TC, Moss SJ and Jurd R (2008). GABA(A) receptor trafficking and its role in the dynamic modulation of neuronal inhibition. Nat Rev Neurosci;9(5):331-43.
- Jarrard LE (1993). On the role of the hippocampus in learning and memory in the rat, Behav. Neural Biol. 60: pp. 9–26.
- Kaech S and Banker G (2006). Culturing hippocampal neurons. Nat Protoc 1:2406–2415
- Kalia M (2005). Neurobiological basis of depression: an update. Metabolism.;54(5 Suppl 1):24-7.
- Kane LP, Lin J and Weiss A: Signal transduction by the TCR for antigen (2000). Curr Opin Immunol, 12:242-249.
- Kaneko M, Stellwagen D, Malenka RC, and Stryker MP (2008). Tumor necrosis factor-alpha mediates one component of competitive, experiencedependent plasticity in developing visual cortex. Neuron; 58, 673–680.
- Kato M, Fukuda T, Wakeno M, Okugawa G, Takekita Y, Watanabe S, Yamashita M, Hosoi Y, Azuma J, Kinoshita T and Serretti A (2009). Effect of 5-HT1A gene polymorphisms on antidepressant response in major depressive disorder. Am J Med Genet B Neuropsychiatr Genet;150B (1):115-23.
- Katz LC, and Shatz CJ (1996). Synaptic activity and the construction of cortical circuits. Science.;274(5290):1133-8.

- Kaur C and Ling EA (2009). Periventricular white matter damage in the hypoxic neonatal brain: role of microglial cells. Kaur C, Ling EA. Prog Neurobiol.;87(4):264-80.
- Kelly KM, Gross RA and Macdonald RL (1990). Valproic acid selectively reduces the low-threshold (T) calcium current in rat nodose neurons. Neurosci Lett 116(1–2):233–238.
- Kessler RC, Berglund P, Demler O, Jin R, Merikangas KR and Walters EE (2005). Lifetime prevalence and age-of-onset distributions of DSM-IV disorders in the National Comorbidity Survey Replication. Arch Gen Psychiatry;62:593–602.
- Kessler RC, Amminger GP, Aguilar-Gaxiola S, Alonso J, Lee S and Ustun TB (2007). Age of onset of mental disorders: a review of recent literature. Curr Opin Psychiatry;20:359-364
- Kew JN and Kemp JA (2005). Ionotropic and metabotropic glutamate receptor structure and pharmacology. Psychopharmacology 179: 4–29
- Kirkwood A, Lee H-K, and Bear MF (1995). Co-regulation of long-term potentiation and experience-dependent synaptic plastic-ity in visual cortex by age and experience. Nature *375*, 328–331.
- Konarski JZ, McIntyre RS, Soczynska JK and Kennedy SH (2007). Neuroimaging approaches in mood disorders: technique and clinical implications. Ann Clin Psychiatry; 19: 265–277
- Kowatch R A, Fristad M, Birmaher B, Wagner K, Findling R, and Hellander M (2005). Treatment guidelines for children and adolescents with bipolar disorder: Child psychiatric workgroup on bipolar disorder. Journal of the American Academy of Child and Adolescent Psychiatry, 44, 213–235.
- Kristiansen LV, Bakir B, Haroutunian V and Meador-Woodruff JH (2010). Expression of the NR2B-NMDA receptor trafficking complex in prefrontal cortex from a group of elderly patients with schizophrenia. Schizophr Res;119(1-3):198-209.
- Kronmüller KT, Pantel J, Köhler S, Victor D, Giesel F, Magnotta VA., et al (2008). Hippocampal volume and 2-year outcome in depression. Br J Psychiatry; 192: 472–473
- Kruusmägi M, Zelenin S, Brismar H and Scott L (2007). Intracellular dynamics of calcyon, a neuron-specific vesicular protein. Neuroreport.;18(15):1547-51.
- Kubota T, Fang J, Guan Z, Brown RA and Krueger JM (2001). Vagotomy attenuates tumor necrosis factor-alpha-induced sleep and EEG delta-activity in rats. Am J Physiol Regul Integr Comp Physiol 280:R1213–R1220
- Kusuari I, Koyama T and Yamashita I(1994). Serotonin- induced platelet intracellular calcium mobilization in depressed patients. Psychopharmacology 113, 322-327
- Lang U and Frotscher M (1990). Postnatal development of non-pyramidal neurons in the rat hippocampus (areas CA1 and CA3): a combined Golgi/electron microscope study. Anat Embryol (Berl) 181:533–545
- Lang T, Bruns D, Wenzel D, Riedel D, Holroyd P, Thiele C and Jahn R (2001). SNAREs are concentrated in cholesterol-dependent clusters that define docking and fusion sites for exocytosis. EMBO J.;20(9):2202-13.
- Lavenex P, Lavenex PB and Amaral DG (2007). Postnatal Development of the Primate Hippocampal Formation, Dev Neurosci;29:179–192
- Lau P, Amadou C, Brun H, Rouillon V, McLaren F, Le Rolle AF, Graham M, Butcher GW and Joly E (2003). Characterisation of RT1-E2, a multigenic family of highly conserved rat non-classical MHC class I molecules initially identified in cells from immunoprivileged sites. BMC Immunol 4:7
- Law AJ and Deakin JFW (2001). Asymmetrical reductions of hippocampal NMDARI glutamate receptor mRNA in the psychoses, Neuroreport 12, pp. 2971–2974.

- Lee JH, Lee J, Choi KY, Hepp R, Lee JY, Lim MK, Chatani-Hinze M, Roche PA, Kim DG, Ahn YS, Kim CH and Roche KW (2008). Calmodulin dynamically regulates the trafficking of the metabotropic glutamate receptor mGluR5. Proc Natl Acad Sci;105(34):12575-80.
- Lee S, Jeong J, Kwak Yand Park SK (2010). Depression research: where are we now? Mol Brain. 10;3:8.
- Lorenzetti V, Allen NB, Fornito A and Yücel M. (2009). Structural brain abnormalities in major depressive disorder: A selective review of recent MRI studies. J Affect Disord, 117(1-2):1-17
- Louis JC, Magal E, Takayama S, Varon S (1993). CNTF protection of oligodendrocytes against natural and tumor necrosis factor-induced death. Science 259:689–692.
- Low LK and Cheng HJ (2005): A little nip and tuck: axon refinement during development and axonal injury. Curr Opin Neurobiol 15: 549– 556.
- Lu YM, Jia Z, Janus C, Henderson JT, Gerlai R, Wojtowicz JM and Roder JC (1997). Mice lacking metabotropic glutamate receptor 5 show impaired learning and reduced CA1 long-term potentiation (LTP) but normal CA3 LTP. J Neurosci 17:5196–5205.
- Luja´n R, Nusser Z, Roberts JDB, Shigemoto R and Somogyi P (1996). Perisynaptic location of metabotropic glutamate receptors mGluR1 and mGluR5 on dendrites and dendritic spines in the rat hippocampus. Eur J Neurosci 8:1488–1500.
- Luja´n R, Roberts JDB, Shigemoto R, Ohishi H and Somogyi P (1997). Differential plasma membrane distribution of metabotropic glutamate receptor mGluR1_, mGluR2 and mGluR5, relative to neurotransmitter release sites. J Chem Neuroanat 13:219–241.
- Luo L and O'Leary DD (2005): Axon retraction and degeneration in development and disease. Annu Rev Neurosci 28: 127–156.
- Machado CJ, Babin SL, Jackson EF and Bachevalier J (2002). Use of MRI techniques to investigate the maturation of the nonhuman primate temporal lobes. Soc Neurosci 28, p. 183
- MacQueen GM, Yucel K, Taylor VH, Macdonald K and Joffe R (2008). Posterior hippocampal volumes are associated with remission rates in patients with major depressive disorder. Biol Psychiatry; 64: 880–883
- Maj M (2000). The impact of lithium prophylaxis on the course of bipolar disorder: a review of the research evidence. Bipolar Disord 2:93–101.
- Makino H and Malinow R (2009). AMPA receptor incorporation into synapses during LTP: the role of lateral movement and exocytosis. Neuron.;64(3):381-90.
- Malenka RC (1994). Synaptic plasticity in the hippocampus: LTP and LTD. Cell *78*, 535–538.
- Manji HK, Moore GJ, Rajkowska G and Chen G (2000). Neuroplasticity and cellular resilience in mood disorders. Mol Psychiatry 6:578–593.
- Manning JS (2010). Tools to improve differential diagnosis of bipolar disorder in primary care. Prim Care Companion J Clin Psychiatry.;12(Suppl 1):17-22.
- Mao L and Wang JQ (2003). Primary striatal neuronal culture. Methods Mol Med 79:379–386.
- Marchese A, Paing MM, Temple BR and Trejo J (2008). G protein-coupled receptor sorting to endosomes and lysosomes. Annu Rev Pharmacol Toxicol;48:601-29.
- Masu M, Tanabe Y, Tsuchida K, Shigemoto R and Nakanishi S (1991). Sequence and expression of a metabotropic glutamate receptor. Nature 349:760–765.
- Mayberg HS (1997). Limbic-cortical dysregulation: a proposed model of depression. J Neuropsychiatry Clin Neurosci;9:471–481.

- McCullumsmith RE, Kristiansen LV, Beneyto M, Scarr E, Dean B and Meador-Woodruff JH (2007). Decreased NR1, NR2A, and SAP102 transcript expression in the hippocampus in bipolar disorder, Brain Res. 1127, pp. 108–118.
- McKinnon MC, Yucel K, Nazarov A and MacQueen GM (2009). A meta-analysis examining clinical predictors of hippocampal volume in patients with major depressive disorder. J Psychiatry Neurosci; 34: 41–54
- McQuillen PS and Ferriero DM (2004). Selective vulnerability in the developing central nervous system, Pediatr. Neurol. 30, pp. 227–235.
- Mesulam MM (1990). Large-scale neurocognitive networks and distributed processing for attention, language, and memory, Ann. Neurol. 28 (5), pp. 597–613.
- Mnie-Filali O, Lambás-Señas L, Zimmer L and Haddjeri N (2007). 5-HT7 receptor antagonists as a new class of antidepressants.Drug News Perspect;20(10):613-8.
- Mongeau R, de Montigny C and Blier P (1994). Electrophysiologic evidence for desensitization of alpha 2-adrenoceptors on serotonin terminals following long-term treatment with drugs increasing norepinephrine synaptic concentration. Neuropsychopharmacology;10:41–51.
- Moret C and Briley M (2000).The possible role of 5-HT(1B/D) receptors in psychiatric disorders and their potential as a target for therapy. Eur J Pharmacol.:404(1-2):1-12.
- Moretta A, Biassoni R, Bottino C, Pende D, Vitale M, Poggi A, Mingari MC and Moretta L (1997): Major histocompatibility complex class Ispecific receptors on human natural killer and T lymphocytes. Immunol Rev, 155:105-117.
- Mori, H. and Mishina, M. (1995). Structure and function of the NMDA receptor channel. Neuropharmacology 34, 1219–1237
- Mota de Freitas D, Castro MM and Geraldes CF (2006). Is competition between Li_ and Mg2_ the underlying theme in the proposed mechanisms for the pharmacological action of lithium salts in bipolar disorder? Acc Chem Res 39(4):283–291.
- Mulhern RK, Palmer SL, Reddick WE, Glass JO, Kun LE, Taylor J, Langston J and Gajjar A (2001). Risks of young age for selected neurocognitive deficits in medulloblastoma are associated with white matter loss, J. Clin. Oncol. 19, pp. 472–479.
- Murphy TK, Segarra A, Storch EA and Goodman WK (2008).SSRI adverse events: how to monitor and manage. Int Rev Psychiatry.;20(2):203-8.
- Murray C and Lopez A (2002). World Health Report 2002: Reducing Risks, Promoting Healthy Life. Geneva, Switzerland: World Health Organization.
- Nakanishi S (1992). Molecular Diversity of Glutamate Receptors and Implications for Brain Function, Science; 258: 597-603.
- Navinés R, Martín-Santos R, Gómez-Gil E, Martínez de Osaba MJ, and Gastó C (2008). Interaction between serotonin 5-HT1A receptors and beta-endorphins modulates antidepressant response. Prog Neuropsychopharmacol Biol Psychiatry;32 (8):1804-9.
- Nelson CA (1995). The ontogeny of human memory: a cognitive neuroscience perspective, Dev Psychol 31, pp. 723–738.
- Nelson CA (1997). The neurobiological basis of early memory development. In: N. Cowan, Editor, The Development of Memory in Early Childhood, Psychology Press, pp. 41–82.
- Ness JK, Romanko MJ, Rothstein RP, Wood TL and Levison SW (2001). Perinatal hypoxia-ischemia induces apoptotic and excitotoxic death of periventricular white matter oligodendrocyte progenitors, Dev. Neurosci. 23, pp. 203–208.
- Nestler EJ, Barrot M, DiLeone RJ, Eisch AJ, Gold SJ and Monteggia LM (2002). Neurobiology of depression. Neuron; 34:13–25.

- Neumann H, Schmidt H, Cavalie A, Jenne D and Wekerle H (1997). Major histocompatibility complex (MHC) class I gene expression in single neurons of the central nervous system: differential regulation by interferon (IFN)-gamma and tumor necrosis factor (TNF)-alpha. J Exp Med 185:305–316
- Ng WX, Lau IY, Graham S and Sim K (2009).Neurobiological evidence for thalamic, hippocampal and related glutamatergic abnormalities in bipolar disorder: a review and synthesis. Neurosci Biobehav Rev.;33(3):336-54.
- Nonaka S, Hough CJ and Chuang DM (1998). Chronic lithium treatment robustly protects neurons in the central nervous system against excitotoxicity by inhibiting N-methyl-D-aspartate receptor-mediated calcium influx. Proc Natl Acad Sci U S A 95(5):2642–2647.
- Nong Y, Huang YQ and Salter MW (2004). NMDA receptors are movin' in. Current Opinion in Neurobiology; 14:353–361
- Nordanskog P, Dahlstrand U, Larsson MR, Larsson EM, Knutsson L and Johanson A (2010).Increase in hippocampal volume after electroconvulsive therapy in patients with depression: a volumetric magnetic resonance imaging study. J ECT;26(1):62-7.
- Nowakowski RS and Rakic P (1981): The site of origin route and rate of migration of neurons to the hippocampal region of the rhesus monkey. J Comp Neurol 196: 126–154.
- Oygur N, Sonmez O, Saka O and Yegin O (1998). Predictive value of plasma and cerebrospinal fluid tumour necrosis factor-alpha and interleukin-1 beta concentrations on outcome of full term infants with hypoxic-ischaemic encephalopathy. Arch Dis Child Fetal Neonatal Ed 79:F190–F193
- Pałucha A and Pilc A (2002).On the role of metabotropic glutamate receptors in the mechanisms of action of antidepressants. Pol J Pharmacol.;54(6):581-6.
- Park M, Penick EC, Edwards JG, Kauer JA and Ehlers MD (2004). Recycling endosomes supply AMPA receptors for LTP. Science;305(5692):1972-5.
- Perova T, Wasserman MJ, Li PP and Warsh JJ (2007). Hyperactive intracellular calcium dynamics in B lymphoblasts from patients with bipolar I disorder. Int J Neuropsychopharmacol 11(2):185–196.
- Pascalis O and Bachevalier J (1999). Neonatal aspiration lesions of the hippocampal formation impair visual recognition memory when assessed by paired-comparison task but not by delayed nonmatching-to-sample task, Hippocampus 9, pp. 609–616.
- Patterson PH (2009). Immune involvement in schizophrenia and autism: etiology, pathology and animal models, Behav. Brain Res. 204, pp. 313–321.
- Peddie CJ, Davies HA, Colyer FM, Stewart MG and Rodríguez JJ (2008). Dendritic colocalisation of serotonin1B receptors and the glutamate NMDA receptor subunit NR1 within the hippocampal dentate gyrus: an ultrastructural study. J Chem Neuroanat;36(1):17-26.
- Petrini EM, Lu J, Cognet L, Lounis B, Ehlers MD and Choquet D (2009). Endocytic trafficking and recycling maintain a pool of mobile surface AMPA receptors required for synaptic potentiation. Neuron.;63(1):92-105.
- Pilc A, Brański P, Pałucha A, Tokarski K, Bijak M (1998). Antidepressant treatment influences group I of glutamate metabotropic receptors in slices from hippocampal CA1 region. Eur. J. Pharmacol.: 349, 83–87.
- Pisani A, Gubellini P, Bonsi P, Conquet F, Picconi B, Centonze D, Bernardi G and Calabresi P (2001). Metabotropic glutamate receptor 5 mediates the potentiation of N-methyl-D-aspartate responses in medium spiny striatal neurons. Neuroscience 106(3):579–587.

- Popoli M, Gennarelli M and Racagni G (2002). Modulation of synaptic plasticity by stress and antidepressants. Bipolar Disord.;4(3):166-82.
- Prange AJ (1964). The pharmacology and biochemistry of depression. Dis Nerv Syst;25:217–221.
- Putney JW (1999) "Kissin' cousins": intimate plasma membrane-ER interactions underlie capacitative calcium entry. Cell 99(1):5– 8.
- Rada C, Lorenzi R, Powis SJ, van den Bogaerde J, Parham P and Howard JC (1990). Concerted evolution of class I genes in the major histocompatibility complex of murine rodents. Proc Natl Acad Sci USA 87:2167–2171
- Renner M, Specht CG and Triller A (2008). Molecular dynamics of postsynaptic receptors and scaffold proteins. Curr Opin Neurobiol;18(5):532-40. Epub 2008 Oct 23. Review.
- Rice D and Barone SJr (2000). Critical periods of vulnerability for the developing nervous system: evidence from humans and animal models. Environ Health Perspect 108:511–533
- Rigucci S, Serafini G, Pompili M, Kotzalidis GD and Tatarelli R (2010). Anatomical and functional correlates in major depressive disorder: the contribution of neuroimaging studies. World J Biol Psychiatry.;11(2 Pt 2):165-80.
- Rosenzweig-Lipson S, Dunlop J and Marquis KL (2007). 5-HT2C receptor agonists as an innovative approach for psychiatric disorders. Drug News Perspect;20(9):565-71.
- Rosoklija G, Toomayan G, Ellis SP, Keilp J, Mann JJ, Latov N et al (2000). Structural abnormalities of subicular dendrites in subjects with schizophrenia and mood disorders: preliminary findings. Arch Gen Psychiatry 57: 349–356.
- Rutledge T, Cosson P, Manolios N, Bonifacino JS and Klausner RD (1992). Transmembrane helical interactions: zeta chain dimerization and functional association with the T-cell antigen receptor. EMBO J 11:3245–3254
- Safer DJ (1997). Changing patterns of psychotropic medications prescribed by child psychiatrists in the 1990s. Journal of Child Psychopharmacology,7,267–274.
- Safer DJ and Zito JM (2006). Treatment-emergent adverse events from selective serotonin reuptake inhibitors by age group: children versus adolescents. J Child Adolesc Psychopharmacol.;16(1-2):159-69.
- Santarelli L, Saxe M, Gross C, Surget A, Battaglia F, Dulawa S, Weisstaub N, Lee J, Duman R, Arancio O, Belzung C and Hen R (2003) Requirement of hippocampal neurogenesis for the behavioral effects of antidepressants. Science 301(5634):805–809
- Savitz J and Drevets WC (2009). Bipolar and major depressive disorder: neuroimaging the developmental-degenerative divide. Neurosci Biobehav Rev;33(5):699-771.
- Scahill L, Hamrin V and Pachler ME (2005). The use of selective serotonin reuptake inhibitors in children and adolescents with major depression. J Child Adolesc Psychiatr Nurs.;18(2):86-9.
- Scannevin RH and Huganir RL (2000). Postsynaptic organization and regulation of excitatory synapses. *Nat. Rev. Neurosci.* 1, 133–141
- Scarr E, Pavey G, Sundram S, MacKinnon A and Dean B (2003). Decreased hippocampal NMDA, but not kainate or AMPA receptors in bipolar disorder, Bipolar Disord. **5**, pp. 257–264.
- Schildkraut JJ (1965). The catecholamine hypothesis of affective disorders: a review of supporting evidence. Am J Psychiatry; 122:509–522.
- Schmidt-Kastner R and Freund TF (1991). Selective vulnerability of the hippocampus in brain ischemia. Neuroscience;40(3):599-636.

- Seress L (1998): Neuronal connections, cell formation and cell migration in the perinatal human hippocampal dentate gyrus. Cesk Fysiol 47: 42–50.
- Seress L (2001). Morphological changes of the human hippocampal formation from midgestation to early childhood. In: C. Nelson and M. Luciana, Editors, Handbook of Developmental Cognitive Neuroscience, Cambridge:MIT Press pp. 45–58.
- Shaikh SA, Strauss J, King N, Bulgin NL, Vetró A, Kiss E, George CJ, Kovacs M and Barr CL (2008). Association study of serotonin system genes in childhood-onset mood disorder. Psychiatr Genet;18(2):47-52.
- Shalak LF and Perlman JM (2002). Infection markers and early signs of neonatal encephalopathy in the term infant. Ment Retard Dev Disabil Res Rev 8:14–19
- Shatz CJ (1983). The prenatal development of the cat's retinogeniculate pathway. J. Neurosci. *3*, 482–499.
- Shatz CJ (2009). MHC class I: an unexpected role in neuronal plasticity. Neuron.15; 64(1):40-5.
- Shi J, Levinson DF, Duan J, Sanders AR, Zheng Y, Pe'er I, Dudbridge F, Holmans PA, Whittemore AS and Mowry BJ *et al.*, (2009). Common variants on chromosome 6p22.1 are associated with schizophrenia, *Nature* **460**, pp. 753–757.
- Shigemoto R, Kinoshita A, Wada E, Nomura S, Ohishi H, Takada M, Flor PJ, Neki A, Abe T, Nakanishi S and Mizuno N (1997). Differential presynaptic localization of metabotropic glutamate receptor subtypes in the rat hippocampus. J Neurosci 17:7503–7522.
- Shohami E, Ginis I and Hallenbeck JM (1999). Dual role of tumor necrosis factor alpha in brain injury. Cytokine Growth Factor Rev 10:119–130
- Sretavan, DW and Shatz CJ (1986a). Interactions between retinal ganglion cells during the development of the mammalian visual system. Annu. Rev. Neurosci. *9*, 171–207.
- Sretavan DW and Shatz CJ (1986b). Prenatal development of retinal ganglion cell axons: segregation into eye-specific layers. J. Neurosci. *6*, 234–251.
- Stefansson H, Ophoff RA, Steinberg S, OAndreassen OA, Cichon S, Rujescu D, Werge T, O.P. Pietiläinen OP, Mors O and Mortensen BP et al., (2009). Common variants conferring risk of schizophrenia. Nature 460, pp. 744–747.
- Strakowski SM, DelBello MP, Sax KW, Zimmerman ME, Shear PK, Hawkins JM and Larson ER (1999). Brain magnetic resonance imaging of structural abnormalities in bipolar disorder, Arch. Gen. Psychiatry **56**, pp. 254–260.
- Suzuki WA and Amaral DG (1994). Perirhinal and parahippocampal cortices of the macaque monkey: cortical afferents. J Comp Neurol 350:497–533.
- Suzuki WA and Amaral DG. (1994). Topographic organization of the reciprocal connections between the monkey entorhinal cortex and the perirhinal and parahippocampal cortices. J Neuroscience 14:1856–1877.
- Suzuki WA (2007). Making new memories: the role of the hippocampus in new associative learning. Ann N Y Acad Sci 1097:1-11. Review.
- Svenningsson P, Chergui K, Rachleff I, Flajolet M, Zhang X, El Yacoubi M, Vaugeois JM, Nomikos GG and Greengard P (2006). Alterations in 5-HT1B receptor function by p11 in depression-like states. Science; 311(5757):77-80.
- Svenningsson P, Tzavara ET, Qi H, Carruthers R, Witkin JM, Nomikos GG and Greengard P (2007). Biochemical and behavioral evidence for antidepressant-like effects of 5-HT6 receptor stimulation. J Neurosci; 27(15):4201-9.
- Swanson LW (1982). Normal hippocampal circuitry. Neuroscience Research Progress Bulletin, 20, 624-637.

- Swayze VW, Andreasen NC, Alliger RJ, Yuh WTC and Ehrhardt JC(1992). Subcortical and temporal structures in affective disorder and schizophrenia: a magnetic resonance imaging study, Biol. Psychiatry 31, pp. 221–240
- Syken J and Shatz CJ (2003). Expression of T cell receptor beta locus in central nervous system neurons. Proc Natl Acad Sci U S A.;100(22):13048-53.
- Talley AK, Dewhurst S, Perry SW, Dollard SC, Gummuluru S, Fine SM, New D, Epstein LG, Gendelman HE, Gelbard HA (1995). Tumor necrosis factor alpha-induced apoptosis in human neuronal cells: protection by the antioxidant N-acetylcysteine and the genes bcl-2 and crmA. Mol Cell Biol 15:2359–66.
- Tanabe Y, Masu M, Ishii T, Shigemoto R and Nakanishi S (1992). A family of metabotropic glutamate receptors. Neuron 8, 169 (1992); K. M. Houamed et al., Science 252, 1318 (1991).
- Thammaroj J, Santosh C and Bhattacharya JJ (2005). The hippocampus, modern imaging of its anatomy and pathology. Practical Neurology 5: 150–159
- Triller A and Choquet D (2003). Synaptic structure and diffusion dynamics of synaptic receptors. Biol Cell;95(7):465-76.
- Triller A and Choquet D (2005). Surface trafficking of receptors between synaptic and extrasynaptic membranes: and yet they do move! Trends Neurosci;28(3):133-9.
- Toro C and Deakin JFW (2005). NMDA receptor subunit NRI and postsynaptic protein PSD-95 in hippocampus and orbitofrontal cortex in schizophrenia and mood disorder, Schizophr. Res. **80**, pp. 323–330.
- Ugolini S and Vivier E (2000): Regulation of T cell function by NK cell receptors for classical MHC class I molecules. Curr Opin Immunol, 12:295-300.
- Utsunomiya H, Takano K, Okazaki M and Mitsudome A (1999). Development of the temporal lobe in infants and children: analysis by MR-based volumetry, AJNR Am J Neuroradiol **20**, pp. 717–723.
- Van Leeuwen JE and Samelson LE (1999). T cell antigen-receptor signal transduction. Curr Opin Immunol.;11(3):242-8.
- Vargha-Khadem F, Salmond CH, Watkins KE, Friston KJ, Gadian DG and Mishkin M (2003). Developmental amnesia: effect of age at injury. Proc Natl Acad Sci USA 100:10055–10060
- Vartanian T, Li Y, Zhao M, Stefansson K (1995). Interferon-gammainduced oligodendrocyte cell death: implications for the pathogenesis of multiple sclerosis. Mol Med 1:732–743.
- Venters HD, Dantzer R and Kelley KW (2000). A new concept in neurodegeneration: TNF-_ is a silencer of survival signals. Trends Neurosci 23:175–180
- Videbech P and Ravnkilde B (2004). Hippocampal volume and depression: a meta-analysis of MRI studies. Am J Psychiatry 161:1957–1966
- Von Gertten C, Flores Morales A, Holmin S, Mathiesen T and Nordqvist AC (2005). Genomic responses in rat cerebral cortex after traumatic brain injury. BMC Neurosci 6:69
- Walden J, Grunze H, Bingmann D, Liu Z and Dusing R (1992). Calcium antagonistic effects of carbamazepine as a mechanism of action in neuropsychiatric disorders: studies in calcium dependent model epilepsies. Eur Neuropsychopharmacol 4:455–462.
- Wang HY and Friedman E (1999) Effects of lithium on receptor-mediated activation of G proteins in rat brain cortical membranes. Neuropharmacology 38(3):403–414.
- Warner-Schmidt JL, Flajolet M, Maller A, Chen EY, Qi H, Svenningsson P and Greengard P (2009). Role of p11 in cellular and behavioral effects of 5-HT4 receptor stimulation. J Neurosci;29(6):1937-46.

- Wasserman MJ, Corson TW, Sibony D, Cooke RG, Parikh SV, Pennefather PS, Li PP and Warsh JJ (2004). Chronic lithium treatment attenuates intracellular calcium mobilization. Neuropsychopharmacology 29(4):759–769.
- Wekerle H (2002). Immune protection of the brain--efficient and delicate. J Infect Dis;186 Suppl 2:S140-4.
- Wittenberg GM and Tsien JZ (2002). An emerging molecular and cellular framework for memory processing by the hippocampus, Trends Neurosci. 25, pp. 501–505.
- Xu J and Ling EA (1994). Upregulation and induction of major histocompatibility complex class I and II antigens on microglial cells in early postnatal rat brain following intraperitoneal injections of recombinant interferon-gamma. Neuroscience 60:959–967
- Yucel K, McKinnon MC, Taylor VH, Macdonald K, Alda M, Young LT and MacQueen GM (2007). Bilateral hippocampal volume increases after long-term lithium treatment in patients with bipolar disorder: a longitudinal MRI study, Psychopharmacology **195**, pp. 357–367.
- Yudowski GA, Puthenveedu MA and von Zastrow M (2006). Distinct modes of regulated receptor insertion to the somatodendritic plasma membrane. Nat Neurosci.;9(5):622-7.
- Zola-Morgan SM and Squire LR (1986). Memory impairment in monkeys following lesions limited to the hippocampus, Behav Neurosci 100**,** pp. 155–160
- Öngür D and Price JL (2000). The organization of networks within the orbital and medial prefrontal cortex of rats, monkeys and humans, Cereb. Cortex 10, pp. 206–219.