UNIVERSITÉ FRANÇOIS – RABELAIS DE TOURS

ÉCOLE DOCTORALE « Sante, Science Biologiques et Chimie du Vivant »

UNITÉ Inserm 930 – Imagerie et cerveau

ÉQUIPE 4 : Troubles Affectifs

THÈSE

présentée par :

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soutenue le : **17 décembre 2012**

pour obtenir le grade de : **Docteur de l'université François – Rabelais de Tours**

Discipline/ Spécialité : Science de la Vie et de la Santé - Neuroscience

Implication fonctionnelle du récepteur P2X7 dans les mécanismes neuroinflammatoires associés à la dépression: étude préclinique

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BLACK BOARD

We, the sons of chalk and slate
We, who copied Cliché's
onto black boards
all their lives !
We, who could never smoothen their
rough bodies turing into wood
We, who kept on building the walls
with borrowed stones !

(محمود اعوان)

(رات سمندر کھیلے)

بلیک بورڈ

اسیں چاک سلیٹ دے جائے
اساں ساری عمر
بلیک بورڈ تے لیکے اکھر
کاپی کیتے
نہ کیتا کدی لکڑ بندے
پنڈے اُتے رندا
نت منگ منگ اٹاں جوڑ دے رہے
کندھ ٹور دے رہے

Dedicated to my ammi and abbu (parents)…
for their unconditional love!

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Acknowledgments

"Thankfulness brings you to the place where the Beloved lives"

Rumi.

Writing acknowledgements is perhaps the most joyful part of a doctoral project, a fortunate one to get distracted from the total scientific stuff write-up. It made me humble by making me realize how significantly other people have contributed to the productivity of my work. So much so that when I started thinking of people who helped me in one way or the other, I felt like being the smallest part of this journey and the love and affection of colleagues, friends and family members filled the all the places. I feel blessed having been among affectionate team mates and wonderful companions throughout my PhD studies. The only fear was to omit someone important from recognizing their contribution due to the write-up stress and jet lag effect after returning from SFN 2012.

I must, first of all, acknowledge Professor Dr. Vincent Camus for supervising this project, for his enthusiasm and resolute dedication to our research objectives and for his kindness and encouragement that he maintained throughout the course of this thesis. He is someone I envy in terms of his interests and expertise and want to match him one day. Gratitude is also payable to our team leader Professor Catherine Belzung, for her guidance, positivity and care in academic as well as non-academic affairs related to my stay in France. Both of them have been extremely helpful and understanding especially at times when I made mistakes and needed them to be exactly the same.

I am also very thankful to Professor Sophie Layé and Dr. Ipek Yalcin-Christman for accepting to be rapporteurs for my thesis. I am grateful to professor Fareed A. Minhas of Rawalpindi Medical College, Rawalpindi, Pakistan in particular for encouraging me to take this project and then again, for travelling all the way from Pakistan to be a part of the jury of my thesis defense. Thanks are also due to Dr. Olivier Guillin and Dr. Sebastian Roger for being part of the jury.

A significant credit of my survival and success in France goes to my French language teachers. I owe a big thanks to my teacher Farah in Alliance Française Islamabad Pakistan and my teacher and friend Sandra in CAVILAM, Vichy France. Their contribution to my output speaks for itself if I mention my success in doing my research masters in French language in 2009 and continuing into doctorate, without knowing a single word of this language before January 2008.

My colleagues in the team including researchers and PhD students have

played a significant role in the overall productivity of me as a person and my research work as a project. I would never have been able to integrate in the team environment without their facilitation and helpful attitude both at the time of experiments and data interpretation as well as in social gatherings. Dr. Samuel Leman, Dr. Frederic Minier, Dr. Boriana Atanasova and Dr. Pascal Barone, I owe a big thanks to all of you for help and guidance. I also feel obliged to name my fellow doctorate and post-doctorate students individually. I started working with Elsa, Arnaud, Mathieu and Anthony and learnt lot of invaluable experimental techniques from them. Having an English speaking person in form of Petra was an obvious relief for me. I would never forget our "bon ba… écoute" joke and down the hill bike riding at the end of the day. Yann, Wahid and Marine also joined the team later on and kept the environment friendly as well as productive. I am immensely thankful to all of them. My heartiest thanks are for Bruno, Anne-Marie, Sévérine and Maryse for always being there when I needed help with technical issues and equipment.

I am indebted to mention the Higher Education Commission of Pakistan for providing monthly stipend. It was like "one less thing to worry". Also, doctoral and post-doctoral students from Pakistan who studied during this time all over the France and overseas deserve big thanks for guidance and help wherever required through the interactive Yahoo groups and Facebook pages. I take this opportunity to name those individually who shared the burden of my worries during this my stay in Tours including Imran, Haroon, Shoukat, Larbi, Maamer, Maya, Muzammil and Fareed. It would also be injustice not to mention my friends in Paris and elsewhere, including Jai, Salma, Shahzina, Shariq, Abdul Qadir, Shoaib and Naeem for accompanying me through my highs and lows during my stay in France. My friends back in Pakistan also equally deserve to be mentioned including Ubaid, Arif, Salman, Rida, Saleem, Iftikhar, Umer and Khurram, not to forget Zoheb in Austria and Bilal in China respectively, each one of them contributing in their own way to keep me in ease regarding my official as well as personal affairs back home.

The biggest and of course the most significant part of my burden has been shared by my family. My parents, my brother Faisal and my sister Tanvir and their families in Sargodha deserve a big mention for supporting me. Equally deserving to be mentioned is my in-laws family in Rawalpindi particularly my parents-in-law, Tayyeba, Zara, Shehzad and Maryam, Murad, Ayesha and Aafaq, for taking care of me, my wife and my son during all these years. I missed them on festivals and family related occasions but always posed like being strong and brave. I knew that they know inside how much their trust and love mean to me.

My better half Maryum and my son Salaar deserve more credit than I personally do for completion of my thesis. The extent of emotional stability, strength and trust that Maryum put in me kept me cool in all the

circumstances that I have been through. Without her love and affection, it would have been an improbable task. I can't forget the moment when I had to come back to France, Salaar was one month old and I had to leave both of them on their own. With her post-graduation studies and Salaar, she managed her worries and happiness without me. Maryum, thanks for walking with me each step of this long expedition. Thanks for travelling to France to be with me in the on the last leg of my thesis journey as well. Yours and Salaar's presence means a lot to me. I would never be able to put it in words what I owe to you. I could only get glimpses of Salaar growing up through Skype which must have led him to believe that his father lives inside a laptop. Fortunately it was over even before thesis itself when I hugged him as he screamed running towards me "Baba I have come to see you in your France". Some moments in life are just priceless, like this one.

In the end, I thank all of those who contributed to my life and my work during these years in one way or the other and whom I couldn't mention in this section.

Résumé

La dépression majeure est un problème préoccupant de santé publique compte tenu de son effet d'augmentation de mortalité précoce par suicide, de l'invalidité qu'elle induit, du coût global qu'elle représente pour les systèmes de santé. La dépression est une pathologie qui affecte indistinctement les populations des pays développés et en voie de développement. La dépression majeure est caractérisée par des symptômes tels que l'humeur dépressive, un sentiment d'impuissance et d'inutilité, des troubles des fonctions instinctuelles (sommeil, appétit, libido), des symptômes de ralentissement moteur, des idées et comportements suicidaires. La physiopathologie de la maladie dépressive commence à être mieux connue. Elle fait état de l'implication de plusieurs systèmes de neurotransmission comme la sérotonine ou la noradrénaline, de l'implication de la neurogenèse dans l'hippocampe, mais également de perturbations de l'axe corticotrope. Toutefois, la mise en évidence d'une surexpression des paramètres inflammatoires chez les sujets déprimés, ainsi que la prévalence accrue de symptômes dépressifs chez les patients souffrant de maladies inflammatoires chroniques ou recevant des chimiothérapies à base de cytokines, ont fait également évoquer la possibilité d'une implication des phénomènes inflammatoires dans la physiopathologie de la maladie. L'IL-1β est l'une des principales cytokines impliquées dans le processus inflammatoire. La Libération de l'IL-1β est en partie régulée par les récepteurs P2X7, récepteurs purinergiques exprimé par de nombreuses cellules dont l'endothélium vasculaire et les cellules microgliales. Le lien entre récepteur P2X7 et dépression a été établi par des travaux cliniques qui ont mis en évidence que certains variant du gène P2RX7 sont associés à une vulnérabilité augmentée aux troubles de l'humeur et à certaines maladies neurodégénératives.

Le projet de recherche développé dans ce travail de thèse s'est attaché à caractériser, à l'aide du modèle de stress chronique modéré imprédictible (SCMI) chez la souris, la composante inflammatoire de la maladie dépressive, notamment la neuroinflammation et le rôle du récepteur P2X7 dans cette composante inflammatoire. Dans une première expérience, nous avons testé un groupe de souris soumises au SCMI. Nos résultats suggèrent qu'il existe chez les souris soumises au SCMI -mais pas chez les souris en situation contrôle-, une activation significative des cellules microgliales dans certaines zones du cerveau en particulier dans l'hippocampe, alors qu'aucune modification de la concentration des cytokines circulantes n'est retrouvée. Une deuxième expérience avait pour but de comparer l'effet d'un antidépresseur classique (la fluoxétine) et d'un antagoniste de récepteur P2X7, le Brilliant Blue G (BBG), sur les caractéristiques comportementales, neuroendocriniennes, inflammatoires et immunohistochimiques chez des souris soumises au SCMI. Nos résultats

montrent que les modifications comportementales et le défaut de freination de l'axe corticotrope (évalué par test à la dexaméthasone) induites par le protocole de SCMI, sont reversées par la fluoxétine et par le BBG. Il est également démontré que la fluoxétine augmente la densité des cellules positives au marquage par la double-cortin (témoignant de la neurogenèse) dans le gyrus denté des souris stressées alors que le BBG est dénué de cet effet sur la neurogenèse.

Les résultats de ces travaux mettent en évidence de manière expérimentale qu'il existe une composante neuro-inflammatoire de la maladie dépressive et qu'un antagoniste (peu spécifique) du récepteur P2X7 a des effets comportementaux et neuroendocriniens similaires à ceux d'un antidépresseur de la classe des SSRI sans en avoir l'effet sur la neurogenèse de l'hippocampe. Ces travaux justifient la poursuite de recherches expérimentales sur l'impact de la neuroinflammation dans la dépression et particulièrement sur le rôle de l'interleukine -1β et du récepteur P2X7 dans la réponse aux traitements pharmacologiques de la dépression. Ils pourraient aussi ouvrir la voie à des travaux en clinique sur l'effet antidépresseur des antagonistes des récepteurs P2X7.

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Liste des abréviations

ICD= International Classification of Diseases
WHO= World health organization
MDD= Major Depressive Disorder
GABA= Gamma amino butyric acid
COMT=catechol O-methyltransferase
MAO-A=Monoamine oxidase A
BDNF= brain-derived neurotrophic factor
5-HIAA= Serotonin 5-hydroxyindoleacetic acid
DHEA= Dehydroepiandrosterone
NaC= Nucleus accumbens
fMRI= functional magnetic resonance imaging
HPA= Hypothalamo-pituitary adrenal axis
CRH= corticotropin releasing hormone
ACTH= adrenocorticotrophic hormone
IL-1β= Interleukin-1 β
TNF-α= tumor necrosis factor α
IL-6= Interleukin 6
SSRIs= Selective serotonin reuptake inhibitor
SNRIs= Serotonin noradrenaline reuptake inhibitor
ECT= Electroconvulsive therapy
UCMS= unpredictable chronic mild stress
DST= dexamethasone suppression test
AD= Antidepressant
TLR= Toll like receptors
ATP= Adenosine triphosphate
LPS= Lipopolysaccharide
IDO= indoleamine-2,3-dioxygenase
SNP= Single nucleotide polymorphisms
BBG= Brilliant Blue G
5-HT = serotonin
ACTH = Adrenocorticotropin hormone
BDNF = Brain derived neurotrophic factor
CRF/CRH = Corticotropin-released factor
DEX = dexamethasone
GABA = Gamma-amino-butyric acid
GR = Glucocorticoid receptor
MR = Mineralocorticoid receptor
NA = Noradrenaline
PVN = Paraventricular nucleus
SCIM = Stress Chronique Imprédictible

Liste des publications et communication

1. Farooq, R.K., Isingrini, E., Tanti, A., Le Guisquet, AM., Arlicot, N., Minier, F., Leman, S., Chalon, S., Belzung, C., Camus, V. Is unpredictable chronic mild stress (UCMS) a reliable model to study depression-induced neuroinflammation? *Behav Brain Res* 2012; 231: 130-137.

2. Farooq, R.K., Tanti, A., Roger, S., Belzung, C., Camus V. Pharmacological antagonism of P2X7 receptor and reversal of UCMS induced depressive like behavior and HPA axis alterations in mice. *En preparation*

3. 37th congress of the European association of geriatric psychiatry (EAGP) 2009, Tours, France Endothelial dysfunction and Depression: A research perspective
   Oral Presentation

4. 10e Colloque de la Société des Neurosciences, Marseille, France 2011Unpredictable Chronic Mild Stress induces neuroinflammation in different stress responsive regions of mice brain
   Poster presentation.

5. Neuroinflammation day, INRA de Tours, Nouzilly, France 2011 Role of P2X7 receptors in pathophysiology of depressive illness
   Oral presentation

6. 19th National Conference, Pakistan Psychiatric Society, Lahore, Pakistan 2011 Animal Models of affective disorders: Backbone of research in psychiatry
   Oral presentation

7. *Society for Neurosciences Annual Meeting New Orleans, United States of America October 12-17, 2012.* P2X7 receptor antagonism reverses UCMS induced depressive like behavior changes in mice
   Poster presentation

Foreword

As a young clinician in Rawalpindi General Hospital, I remember my rotation in the Institute of Psychiatry and the feelings that it induced inside my heart. A society where mental illnesses are less understood and patients are often subjected to irrational treatments, becoming part of a team which dedicated itself to the betterment of the fateful psychiatric patients was an exciting experience. Receiving misdiagnosed and maltreated patients in the outdoor and emergency is a frequent happening. Discouraging fact for the psychiatrist is the relapse of symptoms and the failure of antidepressant response, which puts an additional burden on the hospital services. At around the same time, a major earth quake struck northern parts of Pakistan which brought along with other medical and surgical elements, a type of psychiatric crisis. A large number of patients suffered major traumas, disabilities, loss of the loved ones and shock of witnessing the traumatic event and the subsequent economic burden. The population of this region has had big fallout of psychiatric symptoms since then which keeps on getting complicated on account of the limitations of treatment services, compliance and treatment options. The ongoing law and order situation in Pakistan and the neighboring countries has worsened the pre-existing problems of taboos, under diagnosis and lack of access to treatment facilities.

Higher Education Commission of Pakistan offered a scholarship for research studies in France so that students can be exposed to better research environment in developed countries like France and contribute to the improvement of the research culture in Pakistan on their return. Although the scholarship program is open to all subjects, the number of medical graduates opting to research has been little. My pursuit to look at the psychiatric diseases in general and depression in particular on a basic level was inspired by the difficulties and problems that I came across during my medical studies.

The latest findings of inflammatory aspect of depressive illness motivated us to look at its dynamics using the unpredictable chronic mild stress model of depression in mice. The first experiment was designed to explore the evidence of central and peripheral inflammatory alterations induced by exposure of mice to the UCMS model. As the findings confirmed the presence of significant microglial activity in mice brain as a function of stress, we explored the possibility of antagonizing this process by blocking an important element in the inflammatory cascade, the receptor that induces and delivers the activated interleukin-1β. The antagonism of P2X7 receptor, thus, reversed the stress induced behavioral and biochemical changes in mice. This effect was independent of the dynamics of neurogenesis in the mice brain.

An important implication of these results may be the idea that the inflammatory component of depression may be more susceptible to reversal by the reversal of its proper disturbances rather than by focusing on neurogenesis, which has been the center of antidepressant treatment since a long time. Future therapeutics of depressive illness based on newer research may bring remission to a lot of persistently distressed patients.

Introduction

1.1. Major depressive disorder

Major depressive disorder is a psychiatric disorder primarily characterized by low mood, low self-esteem and loss of enjoyment, energy and concentration. First ever description of depressive illness dates back to 5th and 4th centuries B.C. by Hippocrates which is shown in first of a series of four illustration of Albrecht Dürer aimed to depict four different humors, namely melancholic, phlegmatic, choleric and sanguine (Figure 1). Modern description of major depressive disorder, however, includes a host of heterogeneous and sometime contrasting symptoms which may include low mood, motor and thought retardation, excessive guilt, loss or gain of weight and appetite, feeling of helplessness and worthlessness, disturbances of sleep cycle, decrease appetite and libido, and in severe cases suicidal ideation with or without suicidal behaviors. Psychotic symptoms have also been found to be part of the pathology at times. Heterogeneity of symptomatology and latest discoveries of variations in biochemical markers only makes it difficult for psychiatrists to come to a consensus definition, especially in a given situation where definition is solely based on clinical criteria[1]. International classification of diseases and Diagnostic and statistical manual are the two internationally recognized manuscripts which have been trying to build consensus over its definition. Table 1 lists the International Classification of Diseases 10 (ICD-10) diagnostic criteria for mild, moderate and severe depression in general practice which categorizes the illness into mild, moderate and severe depression (Pedersen, S. H., Stage, K. B., Bertelsen, A. *et al*, 2001).

A
The depressive episode should last for at least 2 weeks
There have been no hypomanic or manic symptoms sufficient to meet the criteria for hypomanic or manic episode at any time in the individual's life
The episode is not attributable to psychoactive substance abuse or to any organic mental disorder
B
Depressed mood to a degree that is definitely abnormal for the individual, present for most of the day and almost every day, largely uninfluenced by circumstances and sustained for at least 2 weeks
Loss of interest or pleasure in activities that are normally pleasurable
Decreased energy or increased fatigability
C
Loss of confidence or self-esteem
Unreasonable feelings of self-reproach or excessive and inappropriate guilt
Recurrent thoughts of death or suicide or any suicidal behavior

[1] The ICD 10 Classification of Mental and Behavioral Disorders, Clinical Description and Diagnostic guidelines, World Health organization.

Complaints or evidence of diminished ability to think or concentrate, such as indecisiveness or vacillation
Change in psychomotor activity with agitation or retardation (either subjective or objective)
Sleep disturbance of any type
Change in appetite (decreased or increased) with a corresponding weight change.
Mild depressive episode All A+minimum 2 B+minimum + 2 C
Moderate depressive episode All A+minimum 2 B+minimum + 4 C
Severe depressive episode All A+all B+minimum + 5 C

Table 1: ICD 10 Diagnostic criteria for single or recurrent depressive episode

Its various subtypes include melancholic depression, atypical depression, catatonic depression, postpartum depression and seasonal affective disorders. Vascular or geriatric depression is a newly proposed type of depression. Depression is also a part of many medical illnesses and occurs as a side effect of a number of treatment regimens especially immunotherapy and chemotherapy. Depression has a strong heritable character too.

Depression is also characterized by a propensity to both chronicity and recurrence. As much as 85% of patients with history of a clinically diagnosed major depressive disorder are expected to predict a recurrence within 15 years, female gender, length severity and number of previous episodes being the strongest predictors of the same (Mueller, T. I., Leon, A. C., Keller, M. B. *et al*, 1999a).

As a consequence of that, depressive illness could be defined as an heritable syndrome of affective, behavioral and biological abnormalities characterized by lowering of mood, anhedonia, suicidal ideation, psychomotor retardation, somatic abnormalities, disruption of negative feedback mechanism of hypothalamo-pituitary-adrenal axis and over-expression of pro-inflammatory markers leading to vascular and neurodegenerative complications if untreated. Although not a primary mandate of this thesis, this proposal of rethinking the definition of depression will highlight the importance of the findings of our experiments in characterizing depression. Following review of literature and presentation of our research work, we will reflect on this definition and will make an effort to justify its contents in the section on future perspectives of our work.

Figure 1: Melancholia by Albrecht Durer. This masterpiece, which dates from 1514, was engraved on copper. The Roman numeral 'I' in the title suggests that this is the first of a series of engravings, possibly aimed at depicting the four Hippocratic temperaments — melancholic, phlegmatic, choleric and sanguine — which were associated with the four humors black bile, phlegm, yellow bile and blood, respectively. A subtype of depression, melancholia is the only word used by Hippocrates 2,500 years ago to characterize a disease on the basis of his theory of the four humors that is still used today to describe the same disease according to official diagnostic classification systems. (Credit caption and figure (Wong, M. L. and Licinio, J., 2004).

1.2. Epidemiology: The dilemma of imprecise diagnostic arsenal

Although the data about prevalence of major depressive disorder varies greatly, the gravity of the challenges remains the same throughout developing and developed world. The heterogeneous presentation of this disorder makes it difficult to formulate a standard diagnostic and evaluation protocol which is applicable across the globe. Social and cultural factors greatly alter the presentation of psychiatric diseases and pose a serious problem for the clinician as well as epidemiologist to make an estimate of the situation leading to imprecisions (Bhui, K. and Bhugra, D., 2001). Its symptomatology is so variable that at times, the psychiatrist is unable to label a patient with a proper diagnosis despite fully knowing that he or she is unwell. This raises the question whether the criteria laid down for diagnoses are compulsory for initiation of treatment, and if compulsory, are they clinically and conceptually valid (Lemoine, M., 2012)?

Its incidence varies according to the group of individuals selected. Selection criteria may vary from different age groups, gender, ethnicity, genetic predispositions, state of physical well-being, presence of potential predisposing factors, and previous exposure to specific risk factors among others.

The statistics state that its prevalence roams between 4-20% of the general population and is projected to become 2nd most important cause of disability by the year 2020 (Bakish, D., 2001). According to the data released by the World Health Organization (WHO), depression affects 121 million people worldwide and is the 2nd cause of disability adjusted life years in 15-44 age group (2012). A multicenter study estimated the highest prevalence of depression at 29.5% (cited in (Bhugra, D. and Mastrogianni, A., 2004). One WHO survey report estimated 12 months prevalence of major depressive episode to be around 5.5% in developing countries and 5.9% in developed countries (Kessler, R. C., Birnbaum, H. G., Shahly, V. *et al*, 2010a).

According to a recent survey in European Union, cost of mental disorders was estimated to be 277 Billion Euros, with mood disorders being the second most common type of mental disorders with a prevalence rate of 7.8%, which is in turn dominated by 6.9% prevalence of unipolar depression (Wittchen, H. U., Jacobi, F., Rehm, J. *et al*, 2011). In United States of America, the economic burden of depression rose from 77.4 billion US dollars in 1990 to 83.1 billion US dollars in year 2000 (Greenberg, P. E., Kessler, R. C., Birnbaum, H. G. *et al*, 2003). In Canada, the life time prevalence of major depressive disorder has been estimated to be around 12.2% with increased incidence associated with advancing age in males

who stayed single, establishing marital status as an important risk factor (Patten, S. B., Wang, J. L., Williams, J. V. *et al*, 2006). Depression, in terms of resource expenditure and limitations of socialization, puts a heavy burden on a society (Pincus, H. A. and Pettit, A. R., 2001).

Of more than 1billion population of India, 3-4% suffer from major mental disorders while another 7-10% suffer from minor depressive disorder (Reddy, M. S., 2010). It is important to remember that underdiagnosis of psychiatric diseases in primary care prevails from developing to the developed countries, with some studies reporting it be 50% less than the actual prevalence of the disease (Ballenger, J. C., Davidson, J. R., Lecrubier, Y. *et al*, 2001). This underdiagnosis is also attributed to the differences in presentation of the illness in different societies, which poses problem especially in expatriate communities. People of Indian and Pakistani origins living in United Kingdom, for instance, present with somatic complaints more often than mood alterations associated with underlying depression (Kamaldeep BHUI, 1999;Jacob, K. S., Bhugra, D., Lloyd, K. R. *et al*, 1998). Gender, marital status and economic problems faced by the masses in developing countries affect their mental well-being (Zainab, S., Fatmi, Z., and Kazi, A., 2012).

Centre	Country	Current depression (%)
Santiago	Chile	29.5
Rio de Janeiro	Brazil	15.8
Paris	France	13.7
Manchester	UK	16.9
Groningen	The Netherlands	15.9
Mainz	Germany	11.2
Bangalore	India	9.1
Athens	Greece	6.4
Berlin	Germany	6.1
Ankara	Turkey	11.6
Seattle, WA	USA	6.3
Verona	Italy	4.7
Nagasaki	Japan	2.6
Shanghai	China	4.0
Ibadan	Nigeria	4.2
Total		10.4
Men		6.8
Female		12.4

Table 2: Transcultural variation in the prevalence of depression (WHO and Goldberg & Lecrubler, 1995) (Bhugra, D. and Mastrogianni, A., 2004)

Age is an interesting factor in this regard. While adolescence is itself a risk factor for psychiatric disorders, any additional insults like childhood trauma and interpersonal difficulties etc. make the possibility of a mental illness even more probable. Approximately 3-5% of children, 4-8% of adolescents and about 20% of adults (18 years old) get affected by major depressive disorder (Bhatia, S. K. and Bhatia, S. C., 2007;Cullen, K., Klimes-Dougan, B., and Kumra, S., 2009;Birmaher, B., Brent, D., Bernet, W. *et al*, 2007). MDD touches lifetime prevalence in 33 Million adults in United States of America and 12 months prevalence in 13 Million approximately, which makes it 16.2% and 6.6% respectively (Kessler, R. C., Merikangas, K. R., and Wang, P. S., 2007). An episode of MDD in the fragile age of

adolescence severely affects academic performance of patients in addition to damaging their mental and physical capabilities, self-esteem, socialization as well as relationships, resulting in burden of mortality as well as morbidity much of which is transferred to adulthood (Weissman, M. M., Wolk, S., Goldstein, R. B. *et al*, 1999). A study of Iranian adolescents exposed to prisons found out that over 70% of them had at least one psychiatric diagnosis at a given time (Ghanizadeh, A., Nouri, S. Z., and Nabi, S. S., 2012). Peter M. Lewinsohn, in his review of the incidence of major depression in adolescence has observed that depressive symptoms in this age group are debilitating and they predicts future psychopathology (Lewinsohn, P. M., Rohde, P., and Seeley, J. R., 1998). In the same review, they have observed high percentage of a psychiatric diagnosis in an attempted suicide in this age group (100% of boys and 94% of girls). In their own pursuit of a risk factor in older adolescents, they state it as follows.

> *To summarize our risk factor research, the prototypical adolescent most likely to become depressed is a 16-year-old female who had an early or late puberty. She is experiencing low self-esteem/poor body image, feelings of worthlessness, pessimism, and self-blame. She is self-conscious and overly dependent on others, although she feels that she is receiving little support from her family. She is experiencing both major and minor stressors, such as conflicts with parents, physical illness, poor school performance, and relationship breakups; she is coping poorly with the ramifications of these events. Other psychopathologies, including anxiety disorders, smoking, and past suicidality are probably present.*

Female gender, thus, is at a clear disadvantage in this regard. Although data about an adolescent age group might not differ to a great degree between the two (Lewinsohn, P. M., Rohde, P., and Seeley, J. R., 1998), adult females are at increased risk of having depressive symptoms when compared to their male counterparts. Pubertal period and transition has been found to be especially important risk factor in this age group (Angold, A., Costello, E. J., and Worthman, C. M., 1998). According to National Comorbidity Survey, a population based epidemiological study, lifetime prevalence of major depression in females is twice (21.3%) that of males (12.7%) (Kessler, R. C., McGonagle, K. A., Swartz, M. *et al*, 1993a). The reason for this difference is unknown, yet various explanations have been proposed including better adopting and distracting skills in face of a stressful condition exhibited by males as compared to females (Nolen-Hoeksema, S., 1987) as well as a relatively dynamic female hormonal patterns including variations in reproductive hormones and associated mood changes (e.g. premenstrual syndrome, mood changes during pregnancy, postpartum depression and psychosis and depressive symptoms associated with post-menopausal syndrome) which may be due to alterations in brain sensitivity to stress at various stages of reproductive cycle (Noble, R. E., 2005a). Genetic

predisposition has also been proposed to be responsible for gender differences in incidence and prevalence of depression (Silberg, J., Pickles, A., Rutter, M. *et al*, 1999a).

Recurrence and chronicity are hallmarks of major depressive illness. Seventy percent of adolescents suffer a recurrence of MDD within 5 years of remission while the overall percentage for an MDD recurrence 15 years after remission is 85% (Birmaher, B., Brent, D., Bernet, W., Bukstein, O., Walter, H., Benson, R. S., Chrisman, A., Farchione, T., Greenhill, L., Hamilton, J., Keable, H., Kinlan, J., Schoettle, U., Stock, S., Ptakowski, K. K., and Medicus, J., 2007;Mueller, T. I., Leon, A. C., Keller, M. B. *et al*, 1999b).

As stated earlier, suicidal ideation is one of the major criteria for diagnosis of major depression. Attempted suicide remains one of the most devastating outcomes of untreated depression (Qin, P. and Nordentoft, M., 2005a). Two potentially vulnerable age groups for suicide are under 30 and over 60, the numbers in the former have risen have tripled recently while the latter is also at an increased risk of suicide (Rich, C. L., Young, D., and Fowler, R. C., 1986;Conwell, Y., Duberstein, P. R., Cox, C. *et al*, 1996). There exists a strong association of this heightened suicide rate with increased depressive symptoms as well as the incidence of drug abuse in younger age group, which has risen sharply during last few decades (Isometsa, E., Henriksson, M., Marttunen, M. *et al*, 1995;Rich, C. L., Young, D., and Fowler, R. C., 1986). Attempted suicide has been associated with psychiatric antecedents in as high as 97-100% of instances in some samples (Cheng, A. T., 1995;Foster, T., Gillespie, K., and McClelland, R., 1997). The risk is particularly important shortly after admission to a health care facility, shortly after discharge as well as associated with a short hospital stay (Qin, P. and Nordentoft, M., 2005b).

In contrast to the higher predisposition of female gender to develop psychopathologies when exposed to stress, male gender is strongly associated with the risk of attempting and completing a suicidal event although data regarding differences in unsuccessful suicidal attempts on the basis of gender vary greatly (Moscicki, E. K., 1994;Qin, P., Agerbo, E., Westergard-Nielsen, N. *et al*, 2000;Bjerkeset, O., Romundstad, P., and Gunnell, D., 2008).

Chronic medical co-morbidities add fuel to the fire. Chronic inflammatory and vascular diseases have a bilateral relationship with depressive illness (Szczepanska-Sadowska, E., Cudnoch-Jedrzejewska, A., Ufnal, M. *et al*, 2010) as well as incidence of suicidal events (Shields, L. B., Hunsaker, D. M., and Hunsaker, J. C., III, 2005). Same is the case with patients suffering from cancer or undergoing cancer chemotherapy (Kendal, W. S., 2007). Despite the overwhelming size of the problem, the strategy to prevent people from committing suicide remains largely insufficient in view of small samples of unsuccessful attempters/postmortem specimens, lack of biological or

cognitive markers that can be used to predict suicide as well as difficulties to develop a research model for this problem (De, Leo D., 2002;Jandl, M., Steyer, J., and Kaschka, W. P., 2010).

The particular depressive syndrome experienced by adults older than sixty years of age is often complicated by comorbid medical (especially vascular and inflammatory) and cognitive abnormalities and difficulties are often encountered for diagnosis as well as treatment. Such challenges have made it obligatory to be considered as a separate entity and dedicate special care and research to its understanding (Thuile, J., Even, C., and Guelfi, J. D., 2007;Kastenschmidt, E. K. and Kennedy, G. J., 2011;Camus, V., Kraehenbuhl, H., Preisig, M. *et al*, 2004a;Blazer, D. G. and Hybels, C. F., 2005). The differences of prevalence across different groups of individuals help identify risk factors and initiate precautionary measures. Identifying risk factors also helps devise an effective diagnostic approach to combat the challenge and reduce the financial burden of the disease.

Given this varied and imprecise picture of prevalence of depression, a lot of work needs to be dedicated to improve the understanding of the illness as well as to apply this understanding to policy making, formulating strategies to estimate the existing and impending burden and economic fallout of the disease and to be prepared to help cure and rehabilitate the fateful sufferers.

1.3. Etiology: The "million dollar question"

What causes depression is a million dollar question. While we know many factors that contribute to the pathophysiology of major depression, classifying them into causative, predisposing and precipitating ones has not been easy. The more we explore the mechanisms underlying this illness, the more difficult it becomes to harmonize the pathophysiological picture of the disease. While stress, especially early life psychological stress remains an undisputed causative factor in first episode sufferers, genetic predispositions in form of single nucleotide polymorphisms of genes remain the only answer to variations in individual responses to comparable stressful events by different individuals. An overview of the relative contribution of these factors is presented in a bid to understand the above mentioned question. These factors have been divided into predisposing and precipitating factors although certain overlap and ambiguity is bound to prevail.

1.3.1. Predisposing factors

1.3.1.1. Genetic factors

Role of genetic factors in pathophysiology of depressive illness can be discussed under two important headings, first the higher prevalence of major depression among individuals in a fraternity sharing genetic inheritance and second, the genetically determined response to a potentially hazardous psychological response, both in terms of coping skills as well as stress related hormones.

Depression has significant heritability. In the largest study of lifetime prevalence of major depression in Swedish twins in terms of number of participants, monozygotic twins of depression sufferers were found to be at higher risk of developing major depression when compared to same gender dizygotic twins of patients suffering from depression (male-male or female-female) while same gender dizygotic twins had the same disadvantage over different gender dizygotic twins (male-female) of depressed subjects (Kendler, K. S., Gatz, M., Gardner, C. O. *et al*, 2006). This may be attributed to the fact that monozygotic twins inherit the same genetic make-up while dizygotic twins inherit roughly half of the same from their parents. The same study found depression to be heritable at about 37%, citing women at higher risk of inheriting this disorder from their predecessors (Kendler, K. S., Gatz, M., Gardner, C. O., and Pedersen, N. L., 2006). Another similar study recorded depression prevalence at 46% in monozygotic and 20% in dizygotic twins in case one of them was suffering from depression which is much higher than a WHO finding cited early on which found it to be 5.5-5.9% in general public (Kessler, R. C., Birnbaum, H. G., Shahly, V. *et al*, 2010b). The heritable major depression is typically recurrent and disabling with longer episodes and frequent suicidality

(Kendler, K. S., Gardner, C. O., and Prescott, C. A., 1999). Although the relationship between depression and suicidality is disputed when compared to suicidal behavior caused by other factors (Ahrens, B. and Linden, M., 1996;Roy, A., Nielsen, D., Rylander, G. *et al*, 1999), the genetic factors in both cases play a significant role and makes a strong basis for their heritability.

Regarding the mechanism of shared phenomena of faulty response to stress, theories exist about genetic modifications inherited by individuals of the same fraternity which predispose them to a typical psychological and hormonal response, leaving them vulnerable to stressful insults. These genetic modifications can be best described as single nucleotide polymorphisms which impact genes encoding proteins resulting in altered responses to stimuli as well as responses to medications. Take for example cortisol secretion in response to stress and there is an exhausting list of genes and their SNPs which determine how a particular individual responds to stressful stimuli in terms of cortisol secretion. To name a few, they include gene variants of the GABA A receptor, the μ-opioid receptor, the serotonin transporter, serotonin 2A receptor, length polymorphism repeat in serotonin transporter region (5-HTTPLR), catechol O-methyltransferase (COMT), monoamine oxidase (MAO A), the α2-adrenergic receptor, brain-derived neurotrophic factor, the angiotensin-converting enzyme, in the glucocorticoid receptor (GR), the TthIIII, NR3C1-1, ER22/23EK, N363S, BclI and the A3669G, and in the mineralocorticoid receptor (MR), the –2 G/C and the I180V all contribute to alter HPA axis responsiveness at different levels (Derijk, R. H., 2009;McMahon, F. J., Buervenich, S., Charney, D. *et al*, 2006;Haenisch, B., Herms, S., Mattheisen, M. *et al*, 2012). Any combination of these potentially master genes determines the quality of an individual's response to stress and makes us believe that genetic predispositions are by far the most pertinent players in causation of this disease.

1.3.1.2. Early life psychological stress

Exploiting the inherited genetic weaknesses, stress exposure in early life determines the eventual fate of one's mood profile. This gene environment interaction can explain the ambiguities related to differences in individual responses towards daily life stresses. Elevations of stress hormones caused by stressful experiences leave their signatures for rest of the life of a potentially vulnerable individual. Maternal separation in animals is a model for early life adverse events which is related to decreased neurogenesis and perturbation of stress hormone regulation which persists through most of the adult life (Lajud, N., Roque, A., Cajero, M. *et al*, 2012). Functional polymorphisms of serotonin transporter (5-HTT) gene have been found to be the determinants of outcome differences in response to life stressors of comparable severity among different individual (Caspi, A., Sugden, K., Moffitt, T. E. *et al*, 2003). Data about human samples experiencing such adversities has also been explored. In one of such study in human setting,

people experiencing maternal separation in early childhood exhibited an alteration of diurnal cortisol secretion up to their middle age (Kumari, M., Head, J., Bartley, M. *et al*, 2012). Cortisol awakening response was found to be elevated in another such study recruiting postpartum women who reported early life adverse experiences as compared to those who didn't (Gonzalez, A., Jenkins, J. M., Steiner, M. *et al*, 2009). While such events don't elicit the same disabilities in everyone they happen to, the final outcome is again dependent upon subjects' genetic make-up. A recent study to find a genetic basis of long lasting effects of childhood diversity revealed association with single nucleotide polymorphisms of corticotropin releasing hormone receptor 1 gene (Laucht, M., Treutlein, J., Blomeyer, D. *et al*, 2012). Another recent study explored the role of minor T-allele of single-nucleotide polymorphisms in the bicaudal C homolog 1 gene (BICC1) in the pathophysiology of depressive illness. Functional MRI revealed that subjects without any history of early life adversity and carrying a protective T-allele of BICC1 had larger hippocampal volumes when compared to those with history of early life adversity carrying the same allele implying that the protective effect of the said gene is lost if the subject experiences an adverse event in his/her childhood (Bermingham, R., Carballedo, A., Lisiecka, D. *et al*, 2012). Taking into account the effect of psychological or physical stress on gene expression, the diversity between identical twins' responses to stress can be explained through the concept of epigenetics (Mill, J. and Petronis, A., 2007). The neurobiological and hormonal imprints of such adversities are important determinants of an individual's behavior in their adult life as well as in terms of their relationships and professional productivity.

1.3.1.3. Gender and Personality type predispositions

Gender and personality are especially important when we discuss a person's potential vulnerability or resilience to the harmful effects of a given stress. As discussed in previous section, female gender is associated with higher incidence and prevalence of depressive illness as compared to their male counterparts. Various reasons hypothesized by researchers include hormonal variations and mood fluctuations associated with various stages of reproductive life, differences in inherent strategies to cope with stress and genetic predispositions (Kessler, R. C., McGonagle, K. A., Swartz, M. *et al*, 1993b;Mazure C.M., 1998;Noble, R. E., 2005b;Nolen-Hoeksema, S., 1987;Silberg, J., Pickles, A., Rutter, M. *et al*, 1999b). Predisposition to depression also comes from certain personality characteristics and temperament when seen in conjunction with recurrence and severity of depressive episodes (Nery, F. G., Hatch, J. P., Nicoletti, M. A. *et al*, 2009). Cognitive personality characteristics are also among important determinants of the outcome of a stressful life encounter (Mazure, C. M., Bruce, M. L., Maciejewski, P. K. *et al*, 2000). Depressive symptoms are predicted by a person's inherent personality traits and account for much of their susceptibility to withstand or otherwise to a major psychological

challenge (Chioqueta A.P. and Stiles T.C., 2005).

1.3.2. Precipitating factors

1.3.2.1. Psychological Stress

Psychological stress encountered in the adulthood in form of adverse life events is the most familiar of the precipitating factors for major depression. It is perhaps the only thing asked by the clinicians when they interview their patients in a psychiatric outdoor save the family history. It is defined as a situation in which environmental demands exceed the adaptive capacity of an organism resulting in psychological or biological changes that may place it at risk of disease, threatening its homeostasis (Cohen, S., Janicki-Deverts, D., and Miller, G. E., 2007). Although adulthood is not characterized by any developmental fragility as earlier part of childhood, sudden and unpredictable traumatic life events prosper on any ground with seeds of vulnerability in the form of inherited SNPs and personality characteristics. As much as 50-80% of depression sufferers report experiencing a major life event 3-6 months prior to the onset of depressive illness (Monroe, S. M. and Simons, A. D., 1991). The most stressful of life events that may result in precipitation of a depressive episode include death of a child, death of a spouse, marital infidelity or separation, business failure, legal trouble, physical ability and diagnosis of a chronic medical illness although bereavement is the one with highest risk of being followed by an episode of major depression (Mazure C.M., 1998). Concomitant presence of these stressors not only determines the parameters of severity of the disorder but also worsen the prospects of recovery (Mazure C.M., 1998). Outcome in terms of treatment response is also affected by the type of event as better outcome is related to loss of a loved one (an adverse interpersonal event) as compared to loss of business (an adverse achievement event) (Mazure, C. M., Bruce, M. L., Maciejewski, P. K., and Jacobs, S. C., 2000).

Psychological stress in form of stressful life events are more likely to cause unipolar depressive illness (Paykel, E. S., 2003), specifically manifesting cognitive-affective symptoms and suicidal ideation and is less likely to cause somatic symptoms (Monroe, S. M., Harkness, K., Simons, A. D. *et al*, 2001).

1.3.2.2. Chronic medical illness

Apart from the physical dangers and complications, being diagnosed with a chronic medical illness is an event of serious psychological consequences. The level of distress associated with diagnosis of breast cancer is a prime example in this regard (Watson, M., Greer, S., Blake, S. *et al*, 1984;Payne, D. K., Sullivan, M. D., and Massie, M. J., 1996). In cases of disability and dependability, it brings even more stress. Many of the chronic medical conditions demand massive changes in lifestyle so as to cope with their physical demands appropriately. Such changes are quite stressful in the

beginning due to their compulsivity and indispensability. A meta-analysis found adults with diabetes at risk of depressive illness twice more than the general population (Anderson, R. J., Freedland, K. E., Clouse, R. E. *et al*, 2001a). Similar data is available for other chronic disorders although it is difficult to separate the element of stress from metabolic and inflammatory ones in terms of their contribution to the induction of depressive symptoms in these patients. For this reason healthy caregivers of the person suffering from disabling ailment have been explored for being at risk of developing depression. In one of such studies, 30-33% of caregivers of stroke survivors were found to have depressive symptoms over 10 months follow up (Berg, A., Palomaki, H., Lonnqvist, J. *et al*, 2005). These data can help formulate better preventive and therapeutic strategies for patients who are undergoing screening and treatment for such diseases as well as their caregivers.

1.3.2.3. Metabolic, Hormonal & Inflammatory disorders

Diseases arising from metabolic and hormonal abnormalities often include a spectrum of symptomatology which is not very far from depressive illness. The prime example of such interaction is the alterations of hypothalamo-pituitary-adrenal axis regulation and its implications in induction of depression as well as response to antidepressant therapy. In addition to dysregulation of its activation as a part of depressive syndrome, a primary pathology of its hormones causes similar symptoms as those of depression. Cushing's syndrome is the commonest example of such phenomenon in which the prevalence of depression was as high as 54% according to one study (Sonino, N., Fava, G. A., Raffi, A. R. *et al*, 1998) and 57% according to another (Kelly, W. F., 1996). Differences of depression prevalence between men and women have also been proposed to be linked to differences of adrenal and sex hormone secretion regulation (for a review see (Fernandez-Guasti, A., 2012). Similarly, abnormalities of prolactin and thyroid hormones are implicated in the occurrence of suicidal thoughts in psychiatric patients (Pompili, M., Gibiino, S., Innamorati, M. *et al*, 2012). Inflammatory disorders are of important consideration in this regard as well and their role is discussed in detail in the section on neuroinflammation.

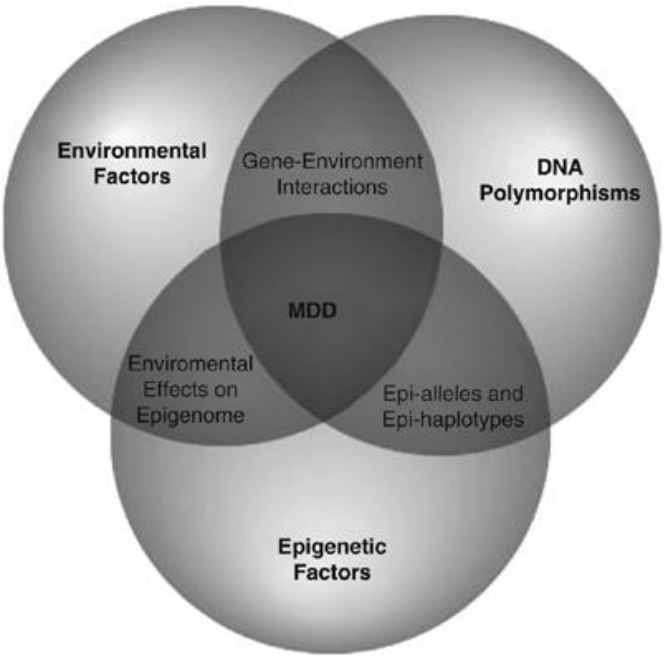

Figure 2: The interaction between genetic, environmental and epigenetic mechanisms i causing MDD (Mill, J. and Petronis, A., 2007)

MDD is thus a product of mutual interaction of genes and environment and subsequent epigenetic phenomena arising from this interaction. An schematic presentation of these interactions has been shown in Figure 2. Knowledge of their mechanisms of interaction as well as their relative contribution to disease pathology in a susceptible group of patients can help us preempt the illness, recognize suitable points of intervention and improve the quality and efficacy of therapeutic interventions which will not only ensure a better outcome but will also save a lot of resources.

1.4. Pathophysiology of depressive illness

Last few decades have been crucial to our understanding of the pathophysiological aspects of depressive illness. During this time period, several hypotheses have been proposed. It can be concluded from the a century's advancement that depression is a heterogeneous illness with far more physical and biochemical abnormalities than initially expected and one may propose to classify patients according to their potential individual predispositions and vulnerabilities in order to find a cure for them, something which highlights our goals in the coming century or so. Major theories proposed to explain this disorder are discussed as follows.

1.4.1. The Chemical Hypothesis: monoaminergic alterations

The monoamine hypothesis of depression has been a center of attention for more than 5 decades (Lee, S., Jeong, J., Kwak, Y. *et al*, 2010). It states that

the brain needs certain monoamines in specific concentration to work appropriately and that deficiency of these chemical compounds lead to a functional deficit that presents with depressive symptoms. The hypothesis itself came into being serendipitously as the antidepressant potential of then used iproniazid was discovered. Although originally used against mycobacterium tuberculosis, it was shown to improve mood symptoms in the patients it was administered to, subsequently observed to improve depression in mycobacterium naïve patients as well (DEVERTEUIL, R. L. and Lehmann, H. E., 1958). Although appreciated for its breakthrough to the understanding of depression pathology, this theory also attracted a lot of criticism for being too simplistic as an explanation to the clinically observed complex behavioral alteration in depressed subjects (Castren, E., 2005a). The hepatotoxic side effects of this compound forced its discontinuation as a drug of choice although other member of this family with fewer side effects were developed and remained under use for long time (Wimbiscus, M., Kostenko, O., and Malone, D., 2010). It was followed by the discovery of antidepressant potential of a tricyclic agent, imipramine. Subsequently many agents with selective and collective potential of increasing monoamine concentrations in the brain were developed the latest of which are selective serotonin and serotonin noradrenaline reuptake inhibitors with varying efficacy which remain drugs of choice in most of the cases so far (Aguglia, E., Ravasio, R., Simonetti, M. *et al*, 2012). It includes widely prescribed Fluoxetine and escitalopram among others (Olfson, M., Marcus, S. C., and Druss, B. G., 2008).

Exploring the mechanisms of their action, it was found that these chemicals raise the concentration of two important neurotransmitters; serotonin and noradrenaline by blocking their re-uptake back to nerve endings or by blocking the enzyme that metabolizes down the amine, called monoamine oxidase, respectively (Slattery, D. A., Hudson, A. L., and Nutt, D. J., 2004;Schildkraut, J. J. and Kety, S. S., 1967;AXELROD, J., HERTTING, G., and POTTER, L., 1962). Additional evidence came from the antihypertensive reserpine and tetrabenazine, which evoked sedation and in some cases severe depression by causing a depletion of catecholamine and 5-hydroxytryptamine stores (LINGJAERDE, O., 1963), which was effectively reversed by earlier mentioned iproniazid and imipramine (BRODIE, B. B., BICKEL, M. H., and SULSER, F., 1961) as well as by a precursor of dopamine as well as noradrenaline called dihydrophenylalanine (DOPA) (Carlsson, A., Lindqvist, M., and Magnusson, T., 1957). Later studies highlighted the independent role of serotonin in mood alteration by showing that up to 20% depletion of noradrenaline alone was unable to induce the sedative effects in animals (Coppen, A., 1967). Depletion of one of the metabolites of serotonin 5-hydroxyindoleacetic acid (5-HIAA) in cerebrospinal fluid alone was found out to have a direct relationship with the possibility of an attempted suicide as well as lethality of the method adopted for suicide

(Asberg, M., Traskman, L., and Thoren, P., 1976).

Blame on alterations in the monoaminergic concentrations and metabolism for being a causative factor in the pathophysiology of depressive illness has been laid due to following reasons. Increase in the monoaminergic metabolism in the depressed populations has been recorded and has been linked either to an increased activity of monoamine oxidase enzyme (Meyer, J. H., Ginovart, N., Boovariwala, A. *et al*, 2006) or decreased activity of these neurotransmitters themselves. Exposure to stress in a laboratory setting has also revealed to have inflicted a deficit of spatial memory, monoamine levels and hippocampal long term potentiation, which were effectively restored by chronic antidepressant therapy (Bhagya, V., Srikumar, B. N., Raju, T. R. *et al*, 2011). Decreased levels of monoamines and dehydroepiandrosterone (DHEA) were observed in nucleus accumbens (Nac), ventral tegmental area (VTA), amygdala and hypothalamus in a rodent model of depression (Malkesman, O., Braw, Y., Ram, E. *et al*, 2008). In yet another study, chronic unpredictable stress in rats was shown to alter monoamine concentrations in hippocampus and limbic structures which also increased oxidative load on the organism adding injury to the insult (Ahmad, A., Rasheed, N., Banu, N. *et al*, 2010).

These findings laid a strong foundation for the monoamine hypothesis of depressive illness, which with the discovery of selective serotonin reuptake inhibitors (SSRIs) and their promising properties of effectivity and safety (Slattery, D. A., Hudson, A. L., and Nutt, D. J., 2004) has kept it alive to date.

Questions were raised following additional research about the validity of the said hypothesis on several bases. First important point was the dietary deprivation of tryptophan which significantly affects the brain concentration of serotonin but doesn't cause neither induction of a depressive syndrome in healthy individuals nor deterioration in depressed unmedicated subjects (Delgado, P. L., 2006). Second point against this hypothesis was the difference in time period required by these drugs in correcting the neurotransmitter concentration and translating it into a behaviorally significant antidepressant effect. Moreover, these drugs do not result in elevation of mood in healthy individuals (Massart, R., Mongeau, R., and Lanfumey, L., 2012;Slattery, D. A., Hudson, A. L., and Nutt, D. J., 2004). In addition side effect profile of these drugs as well as their lack of effect in a majority of the patients has kept the scientists on their toes in the quest for newer and better antidepressants (Montgomery, S. A., Henry, J., McDonald, G. *et al*, 1994;Nemeroff, C. B., 1998).

1.4.2. The Network Hypothesis

To address the potential deficiencies of the chemical hypothesis, abnormalities of networking, information processing and neurotransmission have been proposed to be the cause of mood

alterations.

As Arvid Carlsson, an expert of dopamine and neurotransmission in the brain, stated in his Nobel lecture,

"During the past half-century brain research has been dominated by biochemical approaches, in contrast to the previous half-century, which had a strong electrophysiological emphasis. This switch is understandable in view of the entrance of the neurohumoral transmission concept into brain research in conjunction with the spectacular progress of molecular biology. However, it must be recognized that the brain is not a chemical factory but an extremely complicated survival machine. In order to bring all the forthcoming biochemical observations into a meaningful framework it will prove necessary to emphasize more strongly aspects of neurocircuits"[2]

So, as we can simply put it, brain is a machine which deals with a lot of information processing and this process involves chemicals as well as cellular connections, their maintenance and timely replacement of wear and tear. This ability of the nervous system to adjust and adapt in response to environmental challenges is called neuroplasticity. It includes a number of functional and structural mechanisms that are believed to participate in neuronal remodeling, establishment of information processing networks e.g. formation of new synapses, birth and differentiation of new neurons. A disruption of these mechanisms in the hippocampus, amygdala and cortex has been proposed to be responsible for the mood alterations, cognitive dysfunction, learning and memory impairment and emotional disturbances found in depressive illness, which we term as the network hypothesis of depression. Recent literature is strongly suggestive of the fact that a dysfunction of neural plasticity is a part of the affective disorder pathogenesis (Krishnan, V. and Nestler, E. J., 2008). Although exact mechanisms are not known, it is hypothesized that exposure to stress results in faulty treatment of information due to abnormalities of chemicals as well as networking and which into maladaptive changes in behavior of the subject, resulting in depressive illness. Pharmacological and other forms of therapies aimed at re-establishing the destroyed networks build several new links out of which the stable ones are selected through pruning of synapses and integrated into the existing networks which would lead to improvement in clinical state of the patient, as depicted in Figure 3.

[2]Carlsson, A. A half-century of neurotransmitter research: impact on neurology and psychiatry. Nobel lecture. Nobelprize.org, <http://www.nobel.se/medicine/ laureates/2000/carlsson-lecture.pdf> (2000).

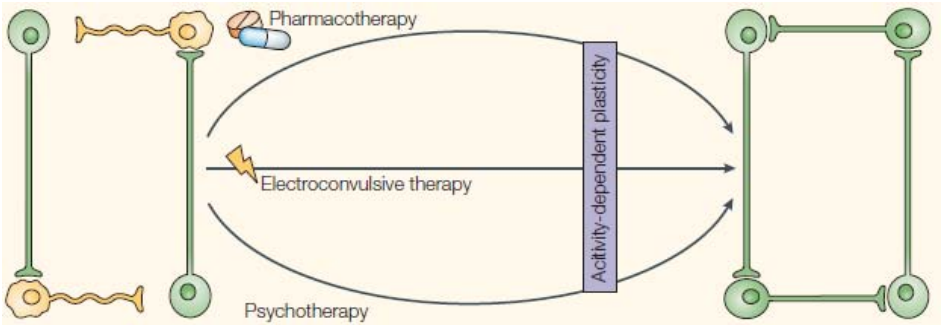

Figure 3: A combinatorial approach for treating depression based on the network hypothesis. Depression might reflect disturbed information processing in neural networks (left panel). A therapeutic regime lead to gradual recovery of network connectivity *(Castren, E., 2005b)*

Neuronal plasticity is intimately linked to cellular responsiveness and is actually the ability of neurons to adapt to changes in their microenvironment, to re-orient them to fight a different opponent, to achieve a new goal. Failure to prove efficiency leaves them vulnerable to environmental challenges, such as stress, which eventually leads to a disease state, i.e. psychopathology. In other words, we can say that some structural and functional entities expose their potential weaknesses to challenging stimuli which leaves them prone to injury and leads to impairment of homeostasis. The extent of injury may vary from minor changes leading to functional compromise i.e. psychiatric illness to major alterations in homeostatic processes resulting in neurodegenerative disorders (Calabrese, F., Molteni, R., Racagni, G. *et al*, 2009). These weaknesses are translated into clinical vulnerabilities in terms of inability to cope with daily life stresses and gives rise to psychopathologies.

Emotional processing in amygdala and other limbic structures is also altered in depressed subjects (Siegle, G. J., Steinhauer, S. R., Thase, M. E. *et al*, 2002) which is susceptible to be restored to normalcy by the antidepressant treatment (Sheline, Y. I., Barch, D. M., Donnelly, J. M. *et al*, 2001), as well as long-term psychotherapy (Buchheim, A., Viviani, R., Kessler, H. *et al*, 2012). An imaging study of suicidal patients revealed smaller right and left orbitofrontal cortex gray matter volume and larger right amygdala volumes when compared to controls which can possibly be implicated in the decision making deficit that these subjects encounter in course of their illness, predisposing them to commit self-harm and subsequent suicide (Monkul, E. S., Hatch, J. P., Nicoletti, M. A. *et al*, 2007). Imaging studies have also shed light on the relative changes in the activity of various cortical and limbic areas corresponding to depressive symptoms and emotional dysregulation underlying depression, (Brody, A. L., Barsom, M. W., Bota, R. G. *et al*, 2001;Beauregard, M., Paquette, V., and Levesque, J., 2006). Modern imaging techniques like ultrasonography, functional magnetic resonance imaging (fMRI) and near-infrared spectroscopy have added beneficial tools to our arsenal of diagnosing major depression related changes in morphology and functional capability of various brain

structures (Becker, G., Berg, D., Lesch, K. P. *et al*, 2001;Desmidt, T., Hachemi, M. E., Remenieras, J. P. *et al*, 2011;Fukuda, M. and Mikuni, M., 2012).

The idea of problematic information processing seems legitimate for many reasons. First, it can explain the latent period of onset of action of antidepressant drugs. As we stated earlier, levels of neurotransmitters are restored much before the antidepressant action of drugs acting on monoaminergic system becomes visible. This time lapse spent in re-establishing the faulty connections is quite understandably the genuine reason of the delay in clinical improvement in depression scores. Support for this idea comes from studying the role of monoamines during the development of brain, specifically in early postnatal life owing to the fact that this is the crucial period for the development and maturation of neuronal networks (Gaspar, P., Cases, O., and Maroteaux, L., 2003). Genetic studies with 5-HT1A modulation in mice indicate that its absence in early postnatal life leads to abnormalities in behavior, and is restricted to early postnatal window (Gross, C., Zhuang, X., Stark, K. *et al*, 2002). Potential role of amines in establishing neuronal networks was reported by a study into kindred of male borderline mentally retard individuals with behavioral alterations including dysregulated impulsive aggression, violence arson and attempted suicide. Subjects were tested for potential changes in monoamine metabolism which revealed interesting findings. The study reported locus for this disorder to be assigned to the Xp11-21 region of X chromosome, in close neighborhood to the genes for monoamine oxidase A and B. First these subjects were reported to have markedly deranged metabolism of monoamines attributed to deficiency of MAOA (Brunner, H. G., Nelen, M. R., van, Zandvoort P. *et al*, 1993). Further research into the same group of individuals revealed a point mutation in the eighth exon of the MAOA structural gene, which changes a glutamine to a termination codon allegedly interfering with the normal development of brain neuronal networks and its behavioral outcome (Brunner, H. G., Nelen, M., Breakefield, X. O. *et al*, 1993). Same pattern of behavioral alterations have been observed in transgenic mice lacking monoamine oxidase A (MAOA) (Cases, O., Seif, I., Grimsby, J. *et al*, 1995) which alters the formation of the cortical somatosensory map proved by the absence of characteristic barrel-like clustering of layer IV neurons in the primary somatosensory cortex and re-established by pharmacological inhibition of 5HT and NA synthesis (Cases, O., Vitalis, T., Seif, I. *et al*, 1996) which further emphasized the role of this system in normal development of brain. Even a pharmacologically conceived increase in serotonin concentration in the developmental phase results in impairment in development of neural circuitry in rats (Xu, Y., Sari, Y., and Zhou, F. C., 2004).

As stated in the section on predisposition to depressive illness, an individual's well-being during the postnatal period is of great importance for the structural and functional development of brain. Exposure to any

stress, physical or psychological, leaves its imprints on the future of the individual. Studies in this regard have found that maternal separation is one of the strongest stresses of early postnatal period. A single 24 hours maternal separation stress on postnatal day 9 was enough to induce depressive like behavior in a strain of mice (Binder, E., Malki, K., Paya-Cano, J. L. *et al*, 2011). Three hours daily maternal separation for first 14 days postnatal leads to a deficit in hippocampal neurogenesis and behavioral modifications in mice with potential long term behavioral and biochemical consequences (Lajud, N., Roque, A., Cajero, M., Gutierrez-Ospina, G., and Torner, L., 2012). Numerous other preclinical and clinical studies exploring vulnerability of hippocampal neurogenesis and other neuroplastic mechanisms to early life stresses have come up with similar results (Teicher, M. H., Anderson, C. M., and Polcari, A., 2012;Hulshof, H. J., Novati, A., Sgoifo, A. *et al*, 2011;Vythilingam, M., Heim, C., Newport, J. *et al*, 2002), which signifies the idea that an insult to the developing brain may disrupt proper networking between different brain areas involved in stress response consequently leading to permanent cognitive and/or behavioral deficit. Adult and late life imaging studies have also reported morphological differences between individuals with respect to their cognitive and behavioral performance (for a review, see (MacQueen, G. and Frodl, T., 2011) . Evidence of morphological changes in brain as a function of stress as well as alterations in the birth and maturation of new neurons makes the network hypothesis capable to answer some of the questions that previously remained unanswered. It should, however, be noted that chemical and network hypotheses are not mutually contradictory. They complement each other to describe the acute and chronic modalities of stress response and pharmacological interventions involving, for example, the SSRIs. This is perhaps the same reason why patients who respond to the available antidepressants in terms of reversal of mood symptoms also show a reversal of the morphological consequences of depressive illness, in addition to the elevation of deranged amine levels (Surget, A., Tanti, A., Leonardo, E. D. *et al*, 2011a;Santarelli, L., Saxe, M., Gross, C. *et al*, 2003).

Main challenges faced by these hypotheses are the unexplained rapid mood elevating effect of deprivation of sleep (Voderholzer, U., 2003) and ketamine treatment (Berman, R. M., Cappiello, A., Anand, A. *et al*, 2000;Zarate, C. A., Jr., Singh, J. B., Carlson, P. J. *et al*, 2006), electroconvulsive therapy (Beall, E. B., Malone, D. A., Dale, R. M. *et al*, 2012), and the non-responsiveness of certain number of patients to the pharmacological spectrum which is potentially capable of reversing chemical as well as neuroplastic aspects of depressive illness (Culpepper, L., 2010).

1.4.3. The Endocrine hypothesis: HPA Axis alterations

The relationship between affective disorders and endocrine alterations was first described by Manfed Bleuler (1903-1994), a Swiss psychiatrist who was son of an eminent psychiatrist Eugen Bleuler (1857-1939). Known to have grown up in the company of schizophrenics, he became interested in psychiatric disorders and their underlying mechanisms which remained his focus of research on his way to become the head of Burghölzli, the Psychiatric Hospital of Zürich, and professor of psychiatry in Zurich. Much of his work is dedicated to the understanding of schizophrenia. In his book *Endokrinologische Psychiatrie* (endocrinological psychiatry), published in 1954, Bleuler presented the idea of psychological aspects of endocrinopathies and their mutual relationship. His research work provided numerous building blocks for an endocrinological branch of psychiatry of the future. Bleuler's description of relationship between psychopathology and endocrine disorders was a major contribution to the understanding of the pathophysiology of psychiatric disorders. His ideas inspired exploration of therapeutic potential of hormone modulation in psychiatry, an approach which is relevant even today (Holsboer, F. and Weber, M. M., 1995).

Although relevance of neuroendocrine secretion to depressive illness in particular was reported in 1962 (GIBBONS, J. L. and McHUGH, P. R., 1962), yet the idea was overshadowed by the euphoria of discovery of antidepressant effects of inhibitors of monoamine metabolism in second half of the 20th century. However, evidence of hormonal aspect of depressive illness continued to amass and was fortified by the discovery of antidepressant modulation of the stress response axis by the conventional antidepressants (for a review see (Holsboer, F. and Barden, N., 1996). The idea of neuroendocrine influence on mood physiology has also remained relevant in light of the unresolved mystery of finding a complete cure for the fateful sufferers of depressive illness.

The neuroendocrine system, also known as hypothalamic–pituitary–adrenal (HPA) axis is a system in which neuroendocrine factors interact with an individual's response to stress. It comprises of hypothalamus, pituitary gland and adrenal glands which communicate with each other through different releasing factors and hormones involved in an individual's response to stress. Briefly, both physical and psychological stressors lead to release of corticotropin releasing hormone (CRH), produced by the hypothalamus. CRH stimulates HPA axis as well as sympathetic nervous system, stimulating release of adrenocorticotrophic hormone (ACTH) and vasopressin (AVP) from anterior lobe of pituitary gland and brings about an increase in blood glucose and heart rate. Animal studies have reported increased blood pressure, increased arousal and vigilance, anorexia, reduced libido, changes in motor activity, and increased tolerance of pain in response to intraventricular administration of CRH

(Sutton, R. E., Koob, G. F., Le, Moal M. *et al*, 1982). ACTH stimulates secretion of glucocorticoids (cortisol in humans and corticosterone in rodents) from adrenal cortex. Glucocorticoids impart a whole bunch of regulatory effects involving both central and peripheral nervous system including coordination of circadian events, such as the sleep/wake cycle and food intake; facilitation of our ability to cope with, adapt to, and recover from stress; and promotion of learning and memory processes through the high affinity type I mineralocorticoid receptors (MR) and the low affinity type II glucocorticoid receptors (GRs). The HPA axis possesses an autoregulatory mechanism mediated by cortisol binding to GRs in the hypothalamus and extrahypothalamic regulatory centers like hippocampus, frontal cortex and pituitary gland (Chrousos, G. P., 1998). This negative feedback control is indispensable to check the excessive secretion of cortisol, in order to maintain homeostasis and avoid the negative effects of its excessive and persistent release. In ideal setting stress response is meant to be of short duration, with increase in cortisol secretion by adrenal medulla supposed to die down in due course of time by exerting a negative feedback effect on secretion of both ACTH and CRH. If this negative feedback control is not functioning properly, cortisol secretion goes unchecked and remains prolonged for excessive periods of time. In depressed subjects elevated levels of CRH and cortisol owing to a dysfunction of HPA axis regulation have been a frequent finding during last couple of decades (Nemeroff, C. B., Widerlov, E., Bissette, G. *et al*, 1984;Rubin, R. T., Poland, R. E., Lesser, I. M. *et al*, 1987;Brown, E. S., Varghese, F. P., and McEwen, B. S., 2004;Bjorntorp, P., 1996) and are considered to exert depressive effects by causing toxic changes in limbic structures including hippocampus (Stokes, P. E., 1995) establishing a cause of the mood alterations (Carroll, B. J., Martin, F. I., and Davies, B., 1968;Plotsky, P. M., Owens, M. J., and Nemeroff, C. B., 1998). The said dysregulation of HPA axis has been reproduced by dexamethasone suppression test (Carroll, B. J., 1984), in clinical settings as well as animal models of depression (Kunugi, H., Ida, I., Owashi, T. *et al*, 2006;Surget, A., Tanti, A., Leonardo, E. D. *et al*, 2011b).

The baseline activity of corticosteroid secretion follows a circadian pattern which is established at around 18 months of age in human offsprings, prior to which unpredictable changes are seen associated with different activities of infancy. This rhythm remains hyper-responsive to stress until 5 years of age (Dettling, A. C., Gunnar, M. R., and Donzella, B., 1999). The stress axis during this period of time remains sensitive to stressful stimuli and its overactivity is thought to be a product of early life events establishing it as a predisposing factor for affective disorders. This is evident from the rodent and non-human primates models that maternal separation of neonates for longer periods stimulates the activity of HPA axis which persists through adulthood similar to the one found in depressed subjects (Sanchez, M. M., Ladd, C. O., and Plotsky, P. M., 2001).

Interpersonal experiences during this sensitive period of time lead to programming the sensitivity of stress response axis which becomes a permanent predisposing factor to dysfunction when stressful situations are encountered in grown up life resulting in psychopathologies (Halligan, S. L., Herbert, J., Goodyer, I. M. *et al*, 2004). Similar findings have been observed in clinical settings, where both men and women with history of early life trauma showed elevated levels of ACTH even in the absence of depressive symptoms; presence of depressive symptoms only deteriorated the elevated ACTH levels (Heim, C., Newport, D. J., Wagner, D. *et al*, 2002;Heim, C. and Nemeroff, C. B., 2002;Heim, C., Mletzko, T., Purselle, D. *et al*, 2008). This would mean that HPA axis alterations may be predisposed by early life events only to be precipitated by stressful events in later life as a part of depressive illness. It may also be assumed that neuroendocrine alterations may only be limited to certain depressed subjects who had been exposed to such stressors in that "vulnerable" part of their development. In the previous section we discussed the effects of maternal separation in early postnatal life which results in disruption of neuronal networks. In addition to the early life events the negative feedback system of HPA axis activation is also influenced by circulating pro-inflammatory molecules (Pariante, C. M. and Lightman, S. L., 2008). In view of the current data, history of such events earlier in life becomes two fold important for being a potential predisposing factor to depressive illness.

The mechanism of HPA axis alterations' interaction with other determinants of an individual's affect like neurogenesis and monoamine alterations is not entirely understood. Glucocorticoid receptors, mainly due to their low affinity, need high concentrations of circulating glucocorticoids to be activated, which in turn makes them involved in stressful conditions when the concentrations of the corticosteroids run high. The high concentration of circulating glucocorticoids is detrimental to neurogenesis (Wong, E. Y. and Herbert, J., 2006). Similarly, reduced GR activity in animal models has been associated with an increased incidence of depressive like symptoms (Chourbaji, S., Vogt, M. A., and Gass, P., 2008). Adverse effects of elevated levels of glucocorticoids on hippocampus occur owing to the presence of highest number of GR and MR receptors. Interestingly, these effects are different in different parts of hippocampus. For example, prolonged elevation of glucocorticoid levels in CA1 and CA3 regions produces morphological changes in pyramidal cells, causing atrophy of their apical dendrites thus compromising their survival and as well as function by hindering their access to incoming information (Magarinos, A. M., Orchinik, M., and McEwen, B. S., 1998). On the other hand, dentate gyrus bears the most devastating effects of unchecked glucocorticoid concentration elevation. It is well established that dentate gyrus is the only part of the hippocampus to host adult neurogenesis; it is also the most sensitive one in terms of negative effects of stress and other challenges. Newborn neurons need a favorable environment for their survival and

maturation, which is drastically affected by high corticosteroid levels. Those cells which somehow survive, suffer damage to their mature phenotypes (Wong, E. Y. and Herbert, J., 2004). On the opposite, adrenalectomy favors proliferation rate of these cells by the virtue of decreased concentration of glucocorticoids (Cameron, H. A. and Gould, E., 1994). An abnormal limbic system drive has also been proposed to be responsible for disinhibition of HPA axis activity (Carroll, Curtis, G. C., and Mendels, J., 1976). Limbic structure have been implicated in mood disorders individually as well as together relating alterations of activity to mood symptoms (for a review see (Herman, J. P., Ostrander, M. M., Mueller, N. K. *et al*, 2005) which make the regulatory effect of limbic stress circuit on HPA axis regulation valuable for future biological and pharmacological research.

Albeit insufficient for a complete efficacy award, conventional antidepressants have been found to exert a regulatory effect on this feedback system by re-establishing the circadian rhythm of glucocorticoid secretion and decreasing their overall concentration (Pariante, C. M., 2006). The underlying mechanism of this interaction and its contribution to behavioral parameters is not fully clear. Nonetheless it does highlight that depressive illness is not a consequence of chemical imbalances alone, but a disease of much more complicated pathophysiology. It is interesting, however, to note that all of the above mentioned hypotheses have been complementary rather than being conflicting to each other.

1.4.4. The vascular hypothesis: Endothelium function alterations

Vascular aspects of depressive illness have come in the limelight mainly due to the epidemiological findings of their co-prevalence. Increasing data suggests a bilateral relationship between the two previously distinct entities. Depressive symptoms predispose individuals to vascular disorders and their presence complicates and worsens the outcome of these disorders. Depressive illness has been found to be associated with increased incidence of ischemic heart disease and myocardial infarction (Hippisley-Cox, J., Fielding, K., and Pringle, M., 1998) as well as increased mortality from cardiovascular disease (Angst, F., Stassen, H. H., Clayton, P. J. *et al*, 2002) (for a systematic quantitative review see (Wulsin, L. R. and Singal, B. M., 2003). Presence of depressive symptoms heralds diabetes (Kawakami, N., Takatsuka, N., Shimizu, H. *et al*, 1999) coronary artery disease as well as stroke (Ohira, T., Iso, H., Satoh, S. *et al*, 2001). Outcome of a cardiac ischemic event is complicated by the presence of depression as an independent risk factor, leading to heart failure as well as death (Williams, S. A., Kasl, S. V., Heiat, A. *et al*, 2002;Penninx, B. W., Beekman, A. T., Honig, A. *et al*, 2001). Clinical data has also linked diabetes mellitis with depressive illness, citing atherosclerosis as a major risk factor (Chen, G., Wu, Y., Wang, T. *et al*, 2012). Diabetic population are twice as likely to suffer from depressive symptoms as compared to general population (Anderson, R. J.,

Freedland, K. E., Clouse, R. E. *et al*, 2001b). Yet another report has cited direct relationship between glycemic profile of patients and their depressive and obsessive symptomatology (Kontoangelos, K., Raptis, A. E., Papageorgiou, C. C. *et al*, 2012). The disease burden of diabetes is on the rise due to macrovascular and microvascular complications associated with depressive illness (Lin, E. H., Rutter, C. M., Katon, W. *et al*, 2010;de Burgos-Lunar, C., Gomez-Campelo, P., Cardenas-Valladolid, J. *et al*, 2012). In the same fashion, depression exhibits a bilateral relation with stroke. Depressed subjects are prone to develop complications of vascular diseases like myocardial infarction and stroke and stroke itself is followed by profound depressive symptomatology (Pariel-Madjlessi, S., Pouillon, M., Robcis, I. *et al*, 2005).

Studies using unpredictable chronic mild stress model of depression in mice have reported an impairment of acetylcholine induced relaxation in aortic vasculature associated with exposure to stress (Isingrini, E., Surget, A., Belzung, C. *et al*, 2011). The same model of depression also reported fluoxetine resistance as a consequence of high fat diet intake in mice, which was also associated with deranged vascular parameters (Isingrini, E., Camus, V., Le Guisquet, A. M. *et al*, 2010).

Depressive symptoms associated with vascular disorders are even more common in older age group, and present a typical entity of depressive illness which is now increasingly being called as geriatric depression (for a review see (Camus, V., Kraehenbuhl, H., Preisig, M. *et al*, 2004b). Relationship between depression and vascular well-being of a patient is thought to be governed by a complex and rather less understood multifactor phenomenon, as described in figure 5 which highlights the factors which constitute risk for both vascular as well as depressive disorder (Camus, V., Kraehenbuhl, H., Preisig, M., Bula, C. J., and Waeber, G., 2004b). It involves interaction between risk factors common to the two groups of diseases. Endothelial dysfunction has been proposed to be the meeting point for these risk factors which may present a therapeutic target for future research and drug development for both depressive and vascular disorders (Isingrini, E., Desmidt, T., Belzung, C. *et al*, 2009;d'Audiffret, A. C., 2010).

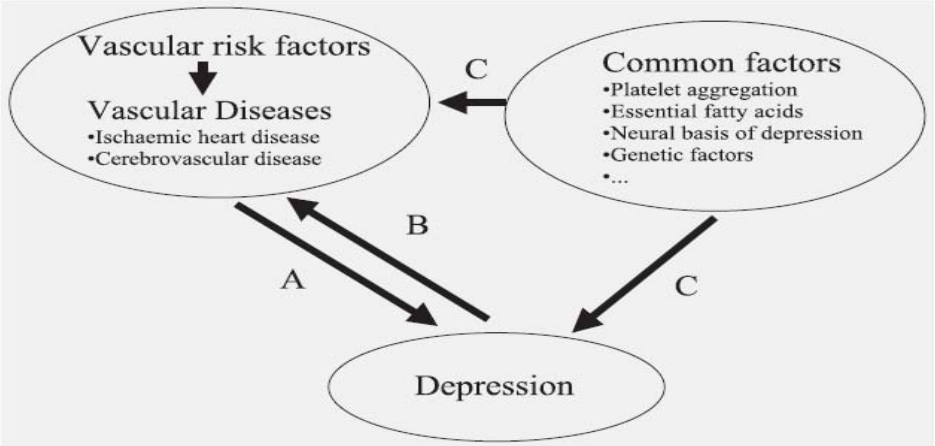

Figure 4: Potential mechanism of association between depression and vascular disease by common risk factors (Camus, V., Kraehenbuhl, H., Preisig, M., Bula, C. J., and Waeber, G., 2004b).

Endothelial dysfunction, as the name implies, is the failure of endothelium to maintain homeostasis. It shifts to a more pro-thrombic and proinflammatory state under the influence of oxidative stress and metabolic and hormonal abnormalities which lead to an imbalance between opposite forces maintaining the optimal level of vasodilatation. This happens due to a reduced liberation of nitric oxide, the principle vasodilator released by endothelium and its consequences may include hypertension and atherosclerosis and in the long run ischemic heart disease, coronary heart disease and stroke, most of them are themselves risk factors for depressive illness (for a review see (Endemann, D. H. and Schiffrin, E. L., 2004). Numerous reports have highlighted endothelial dysfunction as a potential link between stress and vascular disorders (Chen, H., Yiu, K. H., and Tse, H. F., 2011;Do, D. P., Dowd, J. B., Ranjit, N. *et al*, 2010;Sherwood, A., Hinderliter, A. L., Watkins, L. L. *et al*, 2005;Tomfohr, L. M., Martin, T. M., and Miller, G. E., 2008;Wagner, J., Tennen, H., Mansoor, G. *et al*, 2009;Tomfohr, L. M., 2011;d'Audiffret, A. C., Frisbee, S. J., Stapleton, P. A. *et al*, 2010). Stress induced expression of inflammatory determinants has been proposed to induce endothelial dysfunction, independently accounting for as much as 40% cases of atherosclerosis (Black, P. H. and Garbutt, L. D., 2002) an idea which falls well within the existing hypotheses of pathophysiology of depression and is discussed in the following section.

1.4.5. The Immune hypothesis: Neuroinflammatory alterations

The relationship between psychiatric illness and immune system was first observed by an Austrian psychiatrist Julius Wagner- Jauregg (Raju, T. N., 1998). As accidentally as he himself entered the field of psychiatry, Julius observed that patients with general paresis of the insane (GPI), the end stage consequence of syphilis, who develop high grade fever from other causes showed an improvement of their psychiatric symptoms. GPI was considered as a disease with high mortality, severe morbidity, unknown

pathogenesis, imprecise diagnostic features, and no known treatment—in short, a hopeless condition at that time. Soothing words and symptomatic remedy were the only prescription (Raju, T. N., 2006). His observation led him to hypothesize that fever therapy was somehow counteracting the mental decline of the patient. He got encouraging results from his experimental induction of malaria in a group of nine patients with GPI and later reported 83% remission (Wagner-Jauregg, J. and BRUETSCH, W. L., 1946). His results inspired others to follow the pyrotherapy and benefitted thousands of patients. His pioneer work for finding a potential cure by malaria inoculation for a disease which was 'dreadful and incurable' led him to be awarded a noble prize in medicine in 1927. He laid the foundation for consideration of psychiatric illnesses as physical diseases and their interaction with immune system which remain relevant to date (Raju, T. N., 1998).

Although the prime topic of immune interaction with the mood alterations and associated pathologies remained out of limelight during much of the second half of the 20th century, research during last two decades has revolutionized our knowledge about this interaction. Ever since Smith presented his study of cytokine inducing depressive like behavior (Smith, R. S., 1991a), the concept of immune interaction with affect has been given a second life, opening a whole new horizon to explore the etiology and pathogenesis of depression. Since then numerous studies have found links between inflammation and depressive like behavior (also called sickness behavior).

Sickness behavior, first reported by Hart, characterized by hyperthermia, lethargy, sleep and appetite disturbances, and reduced grooming that arise as a consequence of infectious stimulus (Hart, B. L., 1988). Many of these effects were later attributed to interleukin 1, one of various pro-inflammatory cytokines released during the course of infection (Kent, S., Bluthe, R. M., Dantzer, R. *et al*, 1992a). It was reported in the same experiment that central administration of an antagonist effectively inhibited the sickness behavior in an animal in which IL-1β was injected intraperitoneally without inhibiting the hyperthermia and reduction in food motivated behavior. Although Hart noted the whole sickness behavior as an evolutionary strategy to fight against disease yet the bidirectional relationship between cytokines and lowering of mood was enough to lead us to a new dimension in the understanding the pathophysiology of depression. Sickness behavior was also reported by the patients undergoing interferon therapy for viral hepatitis, presenting with low mood, easy fatigability, anorexia, weight loss, altered sleep disturbances, symptoms which are strongly correlated with those of major depression (Papanicolaou, D. A., Wilder, R. L., Manolagas, S. C. *et al*, 1998a;Yirmiya, R., 2000). The experimentators attributed this behavior to the production of pro-inflammatory cytokines as it was inhibited by the administration of

their antagonist.

Depression has been consistently associated with augmented inflammatory markers (Howren, M. B., Lamkin, D. M., and Suls, J., 2009;Dowlati, Y., Herrmann, N., Swardfager, W. *et al*, 2010). Higher number of leucocytes in peripheral blood of depressed subjects (Kronfol, Z. and House, J. D., 1989) and increased plasma and urinary neopterin concentrations (Duch, D. S., Woolf, J. H., Nichol, C. A. *et al*, 1984) which is a very sensitive marker of activation of cell-mediated immunity (Sperner-Unterweger, B., Barnas, C., Fuchs, D. *et al*, 1992) has also been observed as a part of the depression pathophysiology. Neopterin has been proposed to be the link between cell mediated immunity and various depression related symptoms like chronic fatigue and melancholia through activation of oxidative and apoptotic pathways, promoting the expression of proinflammatory markers (Maes, M., Mihaylova, I., Kubera, M. *et al*, 2012). Moreover increased secretion of prostaglandins (Abdulla, Y. H. and Hamadah, K., 1975) and a significantly higher production of IL-18 and IL-6 in culture supernatant of mitogen stimulated peripheral blood mononuclear cells than normal controls (Maes, M., Bosmans, E., Suy, E. *et al*, 1991) has also been reported making the notion of inflammatory aspect of depressive illness even larger. Presence of depressive symptoms in smokers leads to a cumulative pro-inflammatory effect on that individual and predicts worse physical outcome and disability status (Nunes, S. O., Vargas, H. O., Brum, J. *et al*, 2012). Chronic inflammatory conditions like serum lupus erythematosus (SLE) have also been characterized of exhibiting a host of psychiatric symptoms like the challenging high rate of suicide (Fietta, P., Fietta, P., and Delsante, G., 2011). This may be related to the psychological stress associated with chronic illness as well as due to enhanced cellular immunity and other mediators of inflammatory reaction. The increased incidence of depressive symptoms may come from a pre-existing undiagnosed depression, the reduced liberty and increased dependence on others in day to day activities as well as from the knowledge of impending course of disease (the psychological impact of the disease) or the negative effects of the inflammatory by products of disease process on central nervous system. It is, however, difficult to fix the responsibility of a cause and consequence as yet.

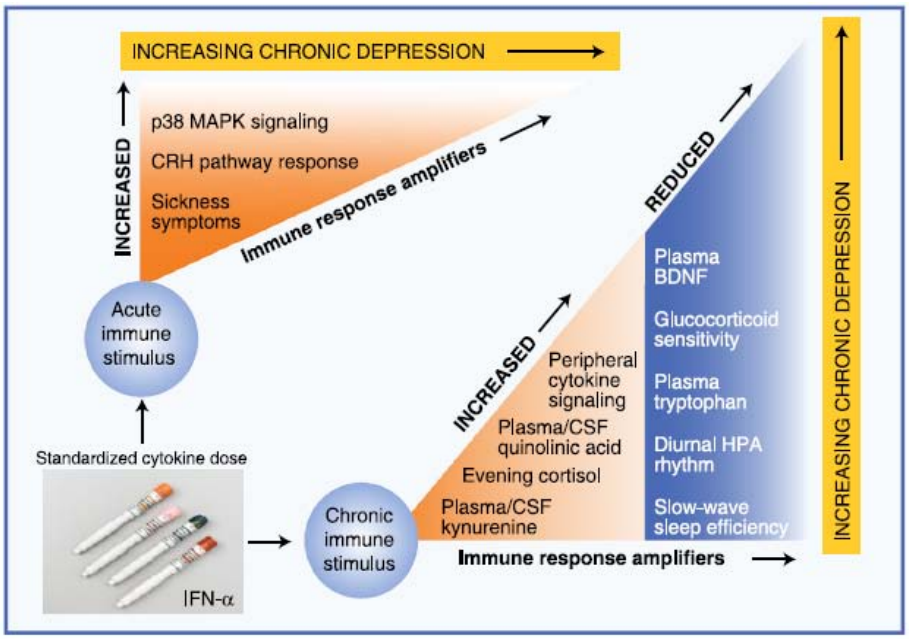

Figure 5: Acute and chronic stimuli and mechanism of their possible contribution to the induction and chronicity of depressive illness (Raison, C. L. and Miller, A. H., 2011).

Depressive episodes have been characterized by enhanced secretion of pro-inflammatory cytokines including IL-1β, TNF-α and IL-6. Depression is a well-known side effect of interferon-α immunotherapy which involves promotion of a host of pro-inflammatory mechanisms including the earlier mentioned cytokines which contribute to depressive symptoms, as shown in figure 6 (Raison, C. L. and Miller, A. H., 2011). Out of these cytokines, IL-6 at the time of admission to hospital was found to be predictive of treatment outcome (Lanquillon, S., Krieg, J. C., Bening-Abu-Shach, U. *et al*, 2000a). IL-6 has also been suggested to be the meeting point of several environmental, immune and genetic factors participating in pathophysiology of depression (Yudkin, J. S., Kumari, M., Humphries, S. E. *et al*, 2000a). IL-6 is also a potent stimulator of HPA axis activation, resulting in highest ACTH production and correspondingly high cortisol secretion (Dentino, A. N., Pieper, C. F., Rao, M. K. *et al*, 1999). IL-1β plays a significant role in stress related neuroinflammatory changes and emergence of depressive symptoms which is discussed in detail in next section *(2.2. Components of neuroinflammatory process).* In addition, proinflammatory cytokines also affect serotonin metabolism, decreasing its availability by stimulating the enzyme indoleamine 2,3- dioxygenase (IDO), which converts tryptophan (Trp), the precursor of 5-HT, into kynurenine (Laugeray, A., Launay, J. M., Callebert, J. *et al*, 2010) which eventually leads to precipitation of depressive symptoms. Inflammatory events reduce the survival and proliferation of new born neurons in the dentate gyrus region of hippocampus, mainly through activation of microglia and production of proinflammatory cytokines, an effect which is reversed by inhibitors of microglial activation (Ekdahl, C. T., Claasen, J. H., Bonde, S. *et al*, 2003a). In addition to this, metabolic changes consistent with inflammatory process like reduced

number of red blood cells, hematocrit and hemoglobin, raised number of reticulocytes and changes in iron metabolism have also been observed in individuals presenting with major depressive illness, comparable with already established markers of inflammation during an episode of depression e.g. lowered serum albumin and zinc (Maes, M., Van, de, V, Vandoolaeghe, E. *et al*, 1996).

The alterations in HPA axis activation through the compromised state of its negative feedback inhibition discussed in previous section have also been associated with increased expression of inflammatory mediators. Exposure to an inflammatory stress in postnatal period can alter the functioning of HPA axis permanently, setting a new threshold of its reactivity for rest of the adult life (Seckl, J. R. and Meaney, M. J., 2004;de Kloet, E. R., Sibug, R. M., Helmerhorst, F. M. *et al*, 2005). Moreover circulating pro-inflammatory cytokines have been shown to activate p38 mitogen activated protein kinase, which enhances the activity of serotonin transporter. Through this and other mechanisms, proinflammatory cytokines contribute to the pathophysiology of depression by altering the availability of serotonin and other monoamines in brain areas related to emotional regulation (limbic system including amygdala, hippocampus and nucleus accumbens) and those related to reward and psychomotor function regulation (mainly the basal ganglia) (Miller, A. H. and Raison, C. L., 2006). The elevated levels of these compounds have been found to be associated with feedback inhibition dysfunction of HPA axis and becomes hallmark of further depression associated consequences (Raison, C. L., Capuron, L., and Miller, A. H., 2006).

Anti-viral interferon alpha therapy gives rise to depressive like behavior in mice as well as humans it is administered to (for a review see (Hayley, S., Scharf, J., and Anisman, H., 2012). The incidence of these symptoms varies from 0% to 70% in different populations (Trask, P. C., Esper, P., Riba, M. *et al*, 2000). Depression with varying severity and associated symptoms like insomnia, irritability, cognitive decline and suicidal ideation can occur with interferon therapy (Yates, W. R. and Gleason, O., 1998;Scheibel, R. S., Valentine, A. D., O'Brien, S. *et al*, 2004;Gitlin, N., 1997). While some authors have believed that incidence of major depression remains the same in patients treated with interferon as compared to the general population (Horsmans, Y., 2005), others believe that major depression can be induced by the treatment, even in subjects without a previous history of major depression (Lotrich, F. E., Rabinovitz, M., Gironda, P. *et al*, 2007). This may lead to a cessation of treatment or reduction of dosage which affects the course of recovery.

The deleterious effects of pro-inflammatory cytokines are numerous. They may affect a large number of brain processes causing altered monoamine and glutamate neurotransmission and reducing adult hippocampal neurogenesis by compromising survival and maturation of new neurons.

They also affect brain signaling patterns and cognition (for a review see (Krishnadas, R. and Cavanagh, J., 2012).

Antidepressants like tricyclic and SSRIs are able to inhibit production of these cytokines and reverse their potential negative behavioral effects as well (Xia, Z., DePierre, J. W., and Nassberger, L., 1996). Imipramine, for instance, has been shown to inhibit cognitive decline by in inhibiting pro-inflammatory cytokines tumor necrosis factor α (TNF-α) in mice (Chavant, F., Deguil, J., Pain, S. *et al*, 2010). Pre-emptive treatment with antidepressants in patients scheduled to undergo interferon alpha treatment for viral hepatitis have also produced encouraging results in many instances (Schaefer, M., Schwaiger, M., Garkisch, A. S. *et al*, 2005;Farah, A., 2002;Goldman, L. S., 1994;Musselman, D. L., Lawson, D. H., Gumnick, J. F. *et al*, 2001). Explaining the effects of immune activation on neurogenesis, metabolism of amines as well as neuroendocrine axis, this hypothesis highlights the complex mechanisms involved in the depression pathophysiology. Still the mysteries of a complete remission in a maximum number of depression sufferers have not been resolved. Many theories regarding the unexplainable lack of response in individuals have been proposed, and newer data exploring antidepressant potential of different candidate agents are being tested with mixed results. Our next section sheds light on the available lines of pharmacotherpeutic strategy for depressive illness. We will also discuss the evolution of chemical compounds with potential antidepressant effects which would be available in near future.

1.5. Therapeutic strategies for MDD

Depressive illness is a complex disorder which exerts physical, psychological as well as metabolic effects on the individual. Severe depression associated with suicidal ideation has significant mortality. This is the reason a comprehensive approach to treat depression is needed (Rihmer, Z. and Gonda, X., 2012), which may include pharmacotherapy, psychotherapy (Tundo, A., Proietti, L., and Cavalieri, P., 2012) and in severe cases, electroconvulsive therapy, repetitive transcranial magnetic stimulation, magnetic seizure therapy, deep brain stimulation, transcranial direct current stimulation, and vagus nerve stimulation (Al-Harbi, K. S., 2012). Some of the treatment strategies for major depressive episodes are summarized here.

1.5.1. Pharmacotherapy

We have come across a long way in the evolution of antidepressant drugs, developing safer and more effective agents as compared to the first generation agents. Several combination therapies have also been suggested although challenges regarding over dosage and toxicity of some of them still remain strong (Wu, M. L. and Deng, J. F., 2011). Ever since discovery of antidepressant potential of modulators of monoamine concentration in brain, these drugs have been the mainstay of pharmacological research. More selective enhancers of concentration of monoamines were developed subsequently which improved efficacy and reduced undesirable side effects of their predecessors. Here we present an overview of the evolution of antidepressant spectrum. Theories regarding origin of these compounds have already been discussed in the section on pathophysiology of depressive illness, so here we will discuss pharmacological aspects of their evolution only.

1.5.1.1. Modulators of Monoamine availability

1.5.1.1.1. Monoamine oxidase A inhibitors

Iproniazid, an inhibitor of monoamine oxidase was used to treat tuberculosis when its antidepressant potential was first observed. Initial research revealed encouraging results regarding their efficacy. Its use, however, was limited in view of its serious side effects e.g. hepatotoxicity. More selective monoamine oxidase A inhibitors were developed. Moreover, reversible inhibitor of monoamine oxidase A, so called RIMA, moclobemide was also evolved which was better than the previous members of its family in terms of its side effects by virtue of which it still hangs around although at some cost of efficacy and selectivity (Amrein, R., Martin, J. R., and Cameron, A. M., 1999;Lotufo-Neto, F., Trivedi, M., and Thase, M. E., 1999).

1.5.1.1.2. Tricyclic antidepressants

Tricyclic antidepressants (TCAs), derived from antihistaminic compounds, have served the purpose of treatment for mood disorders in the middle of

20th century. They inhibit the reuptake of noradrenaline and serotonin thus increasing their concentration. The use of tricyclic antidepressants has considerably dropped since the advent of next generation of antidepressants, the SSRIs although the therapeutic advantage of SSRIs over TCAs has been disputed. A meta-analysis examining 55 double blind studies, for instance, concluded no advantage of newer medication over TCAs. They rather suggested slight benefit in the favor of TCAs for co-morbid depression as well as in hospitalized patients (Anderson, I. M. and Tomenson, B. M., 1994). The side effects of TCAs have been their main drawback with lofepramine being only equally effective but the safest when compared to other members of this group (Davis, J. M., Wang, Z., and Janicak, P. G., 1993).

1.5.1.1.3. Selective Serotonin reuptake Inhibitors

SSRIs are by far the most prescribed drugs for depressive illness in recent times. This class of drugs includes fluoxetine, citalopram, paroxetine, sertraline, fluvoxamine and, more recently, vilazodone (Mandrioli, R., Mercolini, L., Saracino, M. A. *et al*, 2012). These are the safest antidepressants so far and have shown promising results in dealing with multiple aspects of depressive illness e.g. anxiety disorders, comorbid depression and normalization of pro-inflammatory cytokines though data regarding this subject is limited (Hannestad, J., DellaGioia, N., and Bloch, M., 2011). Although their therapeutic efficacy is no more than TCAs, they are certainly better in patient compliance when compared to their predecessors (Anderson, I. M. and Tomenson, B. M., 1995).

1.5.1.1.4. Serotonin Noradrenaline reuptake Inhibitors

Venlafaxine, milnacipran and duloxetine are the flagship antidepressant in this group that block uptake of both serotonin and noradrenaline (Bymaster, F. P., Dreshfield-Ahmad, L. J., Threlkeld, P. G. *et al*, 2001;Romera, I., Perez, V., Manuel, Menchon J. *et al*, 2012). Meta-analyses examining studies reporting efficacy and remission rates amongst patients treated with SSRIs and SNRIs have reported better therapeutic potential of SNRIs (Anderson, I. M., 2001;Thase, M. E., Entsuah, A. R., and Rudolph, R. L., 2001). They have been found to increase proinflammatory cytokines owing to their effect on immune cells (Thayer, J. F. and Sternberg, E. M., 2010).

1.5.1.2. Other agents

Several other agents have been documented to have antidepressant properties with conflicting efficacy compared to current first line treatment choice i.e. SSRIs.

Moclobemide and brofaromine belong to reversible inhibitors of monoamine oxidase A group abbreviated as RIMAs. Meta-analysis examining studies analyzing their relative benefit over other antidepressants have found them only comparable to TCAs in terms of

efficacy but better in side effects profile (Anderson, I. M., 2001). Lithium has also shown some prospect as adjunct treatment strategy in treatment of unipolar depression (Coppen, A., 2000).

Tianeptine, a member of tricyclic group of drugs with a unique function of being a selective serotonin reuptake enhancer, as well as additional effects on glutamate receptors and release of corticotropin releasing factor (CRF) has been found to exert good antidepressant effect and has shown good tolerability in depressed subjects especially with comorbid alcohol addiction (Vukovic, O., Maric, N. P., Britvic, D. *et al*, 2009;Kasper, S. and McEwen, B. S., 2008). In treatment resistant depression, Tianeptine has been found to be of some benefit when administered in combination with other antidepressant medicine (Niederhofer, H., 2003).

Another interesting compound in this regard is ketamine. Used as an anesthetic agent, ketamine has shown to induce rapid and lasting behavioral restitution in treatment resistant major depression sufferers (Murrough, J. W., Perez, A. M., Pillemer, S. *et al*, 2012) subjects as well as in animal models (Autry, A. E., Adachi, M., Nosyreva, E. *et al*, 2011). The mechanism of action is not fully understood but is thought to be antagonism at *N*-methyl-D-aspartate glutamate receptor (NMDA) receptors as well as transient increases in BDNF translation which improvement in synaptic plasticity (Autry, A. E., Adachi, M., Nosyreva, E., Na, E. S., Los, M. F., Cheng, P. F., Kavalali, E. T., and Monteggia, L. M., 2011).

Agomelatine, a melatonergic agonist and 5-HT_{2C} antagonist, has been lauded for its therapeutic potential and safety profile in treating depression, particularly highlighting its safety with over dose, lack of withdrawal syndrome and fewer sexual side effects (Sansone, R. A. and Sansone, L. A., 2011).

St. John's wort, saffron, rhodiola, lavender, echium, and the Chinese formula banxia houpu polygala tenuifolia, the traditional Chinese herbal formula, gan mai da zao, and Cannabis sativa constituents have also been reported to exert antidepressant action to different degrees (El-Alfy, A. T., Abourashed, E. A., and Matsumoto, R. R., 2012).

1.5.1.3. Future candidates

Anti-inflammatory agents have been shown to have some antidepressant potential alone (Tyring, S., Gottlieb, A., Papp, K. *et al*, 2006) as well as in adjunct therapy with conventional antidepressants by shortening their duration of onset of action (Mendlewicz, J., Kriwin, P., Oswald, P. *et al*, 2006;Brunello, N., Alboni, S., Capone, G. *et al*, 2006). When administered in combination with Fluoxetine, cyclo-oxegenase-2 inhibitor celecoxib exhibited a superior antidepressant profile as compared to Fluoxetine with placebo (Akhondzadeh, S., Jafari, S., Raisi, F. *et al*, 2009). Bis-eugenol, an

anti-inflammatory agent has been shown to induce antidepressant effects in mice by promoting monoamine levels in their brains even when its anti-inflammatory effects were blocked (do Amaral, J. F., Silva, M. I., de Aquino Neto, M. R. *et al*, 2012). Modafinil, buspirone, Substance P antagonist, dehydroepiandrosterone (DHEA), orexin receptor blockers, melanin concentrating hormone, *alpha*-amino-3-hydroxy-5-methyl-4-isoxazole propionate (AMPA) receptor antagonists and several other chemical agents are being tested for their antidepressant potential. Along with these, other agents with potential antidepressant effects on different systems participating in the pathophysiology of major depression will be developed to improve efficacy and tolerability and to reduce latency of onset of action.

1.5.2. Psychotherapy

Psychotherapy, much like pharmacotherapy can be considered as first line treatment strategy for mild to moderately depressed subjects (Casacalenda, N., Perry, J. C., and Looper, K., 2002). Several studies have reported its efficacy in treatment of depressive illness (Cuijpers, P., Dekker, J., Hollon, S. D. *et al*, 2009;Keller, M. B., McCullough, J. P., Klein, D. N. *et al*, 2000). Its combination with newer antidepressants remains to be tested and offers a hope for the fateful sufferers.

1.5.3. Electroconvulsive therapy

Electroconvulsive therapy (ECT) was introduced into treatment of mood disorders in 1938, four years after the discovery of therapeutic chemical convulsions. Countless theories implying psychological, neurophysiological and neuroplastic changes have been proposed yet the exact mechanism of its antidepressant action remains largely unknown (Sandeep Grover, Surendra Kumar Mattoo, and Nitin Gupta, 2005). Its efficacy in all types of depression particularly treatment resistant depression, however, remains undisputed (Pagnin, D., de, Queiroz, V, Pini, S. *et al*, 2004;Husain, M. M., McClintock, S. M., Rush, A. J. *et al*, 2008;Kho, K. H., van Vreeswijk, M. F., Simpson, S. *et al*, 2003). Suicide, a possibly fatal complication of an untreated major depressive episode is an established indication for electroconvulsive therapy (Prudic, J. and Sackeim, H. A., 1999). An acute intervention by ECT is followed by a maintenance therapy including first line antidepressants as well as mood stabilizer agents (Coppen, A., 2000). Use of ECT in third world countries, however, remains limited due to lack of knowledge in general public and its apparent invasive nature (Arshad, M., Arham, A. Z., Arif, M. *et al*, 2007).

1.6. Challenges regarding treatment of major depressive disorder

Of course the biggest challenge to-date for neuroscience is to find a way to complete cure of major depressive disorder. Achieving it has been an uphill task. Despite extensive neurobiological and pharmacological research in last couple of decades, initial treatment response rate has remained at about 63-64% and complete remission has only been achieved in less than half of the patients treated with at least two different antidepressants (Thase, M. E., Haight, B. R., Richard, N. *et al*, 2005). Switching to a different antidepressant monotherapy or in combination with other drugs may benefit those who fail to respond appropriately to the initial first line medication (Marangell, L. B., 2001). Treatment resistance, thus, represents a major obstacle to realize the dream of finding a cure for MDD (Sackeim, H. A., 2001).

Many a times, treatment has to be withdrawn or changed due to appearance of undesirable effects. Monoamine oxidase inhibitors, the earliest antidepressants lost being the favorites with the discovery of their side effects related to hepatotoxicity. Tricyclic compounds had to be discontinued due to many cases of tyramine-induced hypertensive crisis were reported allegedly happening after intake of tyrosine rich compounds such as cheese hence called the cheese reaction (HORWITZ, D., LOVENBERG, W., ENGELMAN, K. *et al*, 1964;ASATOOR, A. M., LEVI, A. J., and MILNE, M. D., 1963). The limitations on choice of food made it impossible for many people to take these medicines. Nonselective and reversible compounds were developed subsequently at the cost of efficacy and specificity, of which only moclobemide remains around to-date (Lotufo-Neto, F., Trivedi, M., and Thase, M. E., 1999). Globally, they remain underused due to diet restriction associated with their use (Wimbiscus, M., Kostenko, O., and Malone, D., 2010).

Tricyclic antidepressants had their own side effects which included weight gain, dry mouth, (Berken, G. H., Weinstein, D. O., and Stern, W. C., 1984). Sexual dysfunction has been associated with SSRIs (Atmaca, M., Korkmaz, S., Topuz, M. *et al*, 2011). SSRIs as well as the newer SNRI group agent Venlafaxine have been associated with a withdrawal syndrome upon abrupt discontinuation (Sabljic, V., Ruzic, K., and Rakun, R., 2011). In view of these and other side effects, many depressed subjects fail to show either primary response or to sustain the therapeutic benefit of available antidepressant spectrum (Papakostas, G. I., 2008). These challenges have kept the scientists guessing if a pharmacological compound with properties of complete and definitive remission is possible to develop or not. In other case depression might need to be broken into different types according to their pathophysiological picture and treated individually with different compounds.

1.7. Building an animal model of depression vulnerability

Nothing beats the idea of a purposely constructed animal model to understand and explore pathophysiology of an illness and test a therapeutic intervention for the same. Diversity of life on this planet enables us to conceive variety of non-human hosts for human diseases such as rodent and non-human primate. An animal model is defined as a controlled induction of an experimental situation developed to study a particular condition or phenomenon in the same or different species. While it is easy to model a physical illness e.g. infection in an animal, building an animal model of a psychiatric illness is not a piece of cake. The prime reason for this is the fact that psychiatric illnesses involve perturbations of the very psychological parameters which distinguish us, the humans, from animals. Emotions in humans, for example, are far better developed than any of the living species on this planet. Same is the case for cognition, learning and memory. Second important reason behind the difficulty related to modeling psychiatric disorders is our incomplete connaissance of their proper pathophysiology. While it is obvious that the purpose of establishing models is to increase our understanding of these phenomena, it is hindering us to go to the next step of using an ideal model to find the moment of an intervention to prevent or treat it. Lastly, the symptoms observed in humans as a function of their low mood including hallucinations, delusions, sadness and guilt are either unknown to exist in lower animals or impossible to recognize and evaluate so far (Nestler, E. J. and Hyman, S. E., 2010). Nevertheless last couple of decades has seen great advancements in this field and with advances in experimental techniques and equipment, animal models will be more and more productive and contributive to the overall understanding of pathophysiology of depressive illness.

1.7.1. Validation criteria of Paul Willner

Many criteria for modeling a human disease in animals have been suggested in last century. The most reliable and the most referred to for a psychiatric disease are the three validation criteria proposed by Paul Willner (Willner, P., 1986;Willner, P., 1984a) to evaluate an existing animal model of human disease and also to develop a new model.

1.7.1.1. Face validity

Willner described face validity of an animal model of human disease as its similarity of clinical presentation in humans, emphasizing on 'no more no less' picture of depression. At the same time, it also stresses on exactness of symptomatology of the disease.

1.7.1.2. Predictive validity

Predictive validity of an animal model of depression may refer to the

similarity of response to a therapeutic intervention. Initially, only pharmacological interventions were included in the definition but later on, non-pharmacological intervention such as electroconvulsive therapy was also included (Belzung, C. and Lemoine, M., 2011a).

1.7.1.3. Etiological/Construct validity

In Willner's own words, 'construct validity correspond to the fact that 'both the behavior in the model and the features of depression being modeled can be unambiguously interpreted, and are homologous, and whether the feature being modeled stands in an established empirical and theoretical relationship to depression (Belzung, C. and Lemoine, M., 2011a).' In a relatively simpler fashion, Willner described this later on as the characteristic of an animal model which would mean sharing a human symptom of the disease being modeled as well as sharing the mechanism by which the disease process led to that particular symptom, which is relatively close to other authors defining the same criterion (Epstein, D. H., Preston, K. L., Stewart, J. *et al*, 2006). This part of the validation criteria is by far the most difficult one to be certain of in many animal models of human diseases. This is because course of pathology is the least known variable. It is usually based on hypotheses and theories relying on whatever knowledge we have concerning the physiological and pathological phenomena of the illness under study hence remains mostly conceptual (Chadman, K. K., Yang, M., and Crawley, J. N., 2009).

1.7.2. Validation criteria of Belzung & Lemoine

Following the landmark criteria proposed by Willner, many authors have used them to evaluate different models of affective disorders. These criteria have also been subjected to a lot of revision and discussion. Belzung and Lemoine have recently proposed nine criteria undertaking the three proposed by Willner et al. breaking them into detail and adding few new ones (Belzung, C. and Lemoine, M., 2011b). The higher number of parameters suggested by Belzung and Lemoine doesn't necessarily break the three Willner's criteria into sub-categories but adds new ones as well, underlining the newer advancements of our understanding of the mechanisms involved in induction of depressive illness. The nine criteria are described in the figure 7 below.

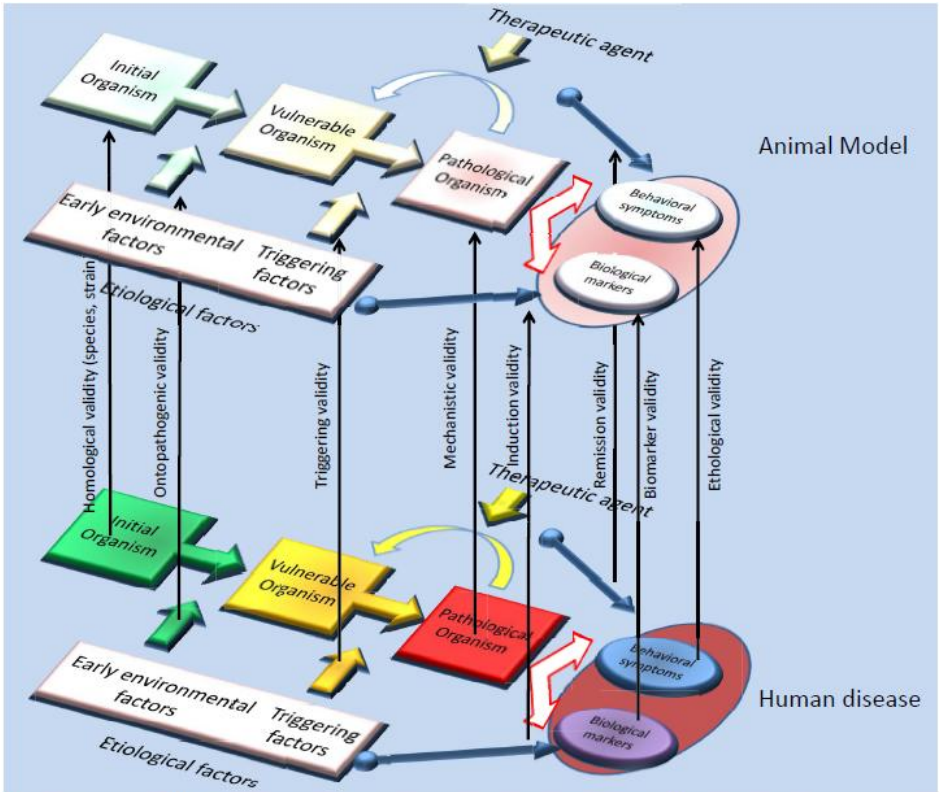

Figure 6: Criteria of validity for animal models as proposed by Belzung and Lemoine. The figure depicts a step to step similarity of disease components in humans and their possible reproduction in animal models (Belzung, C. and Lemoine, M., 2011a).

There are four major criteria in this proposal with each one of them divided into two important categories. They include homological validity (including species and strain validity), pathogenic validity (which includes ontopathogenic and triggering validity), mechanistic validity, face validity (comprising of ethological as well as biomarkers validity) and predictive validity (induction validity, remission validity). These criteria have incorporated the latest developments in depression pathogenesis and treatment outcomes, labeling different models of depression for their respective validity and acceptability.

Based on these criteria, various animal models of depression have been proposed which are summarized in the following table (adopted from Jan M. Deussing 2006). The unpredictable chronic mild stress in mice, the model used for experimentation during the research work carried out for this thesis is an adaptation of chronic mild stress model in rats and is discussed in detail in the following section

Model	Comments
Stress in adulthood	
Learned helplessness	Sensitive to some antidepressants Sensitive to acute treatment only Requires very strong stressors Ethical restrictions
Chronic mild stress	Sensitive to chronic antidepressants Poor reproducibility Only some antidepressants are effective Labor intensive
Social stress	Sensitive to chronic antidepressants Poor reproducibility Only some antidepressants are effective Labor intensive
Early life stress	
Maternal deprivation	Effects of antidepressants are uncertain
Lesions	
Olfactory bulbectomy	Sensitive to chronic antidepressants Mechanism of action uncertain Invasive
Pharmacological	
Reserpine	Sensitive to antidepressants Mood-lowering effect is unclear Nonselective for all monoamines
Tryptophan	Sensitive to antidepressants Does not model depressive signs or symptoms
Psychostimulant withdrawal	Causes depression-like symptoms Sensitive to antidepressants Symptoms are only transient ~1 week Labor intensive Needs further validation
Genetic	
Genetically engineered mice	Allows to study single gene Endophenotype interaction No single vulnerability gene available Cannot model multigenic diseases
Selective breeding	Individual differences in Susceptibility to depression, Can model multigenic diseases Further validation needed

Table 3: Comparison of different animal models of depression and their validity characteristics

1.7.3. Unpredictable Chronic Mild Stress (UCMS)

Willner described chronic unpredictable stress model as one of the highest scoring model based on his own criteria (Willner, P., 1984b). The scoring and comparison of various models assessed by Willner is given in the table 3. Later on, he described a chronic mild stress model of depression rats (Willner, P., Muscat, R., and Papp, M., 1992)and in a 10-years follow up review, he described it as better than most other models on the given criteria of validity (Willner, P., 1997).

Model	Validity		
	Predictive	Face	Construct
Group 1 (Good?)			
14. 'Behavioural despair'	++	+	
15. Chronic stress	++	++	+
16. Separation	+	++	+
18. Self-stimulation	++	+	++
Group 2 (Interesting)			
3. Yohimbine (dogs)	++		
10. Chronic isolation	+++		
11. Exhaustion stress	+	+	
12. Circadian rhythms	++	+	
17. Incentive disengagement		+	
Group 3 (Problematic)			
1. Muricide	+		
9. Olfactory bulbectomy	+	+	
13. Learned helplessness	++	+	+
Group 4 (Poor)			
2. Yohimbine (mice)			
4. Dopa potentiation			
5. Kindling			
6. Reserpine reversal			
7. Amphetamine potentiation			
8. 5-HTP reversal			

Table 4: Validity scores of differnt animal models of depression according to Willner. Note that chronic mild stress has one the highest scores of validity as a true projection of human disease in animals

A variation of this model by the name of unpredictable chronic mild stress (UCMS) was tested in different strains of mice with a range of antidepressants. It identified inbred BALB/c mice to be the most vulnerable one to this stress protocol as well as responsive to the drug treatment with imipramine, a mixed serotonergic–noradrenergic reuptake inhibitor, desipramine, a more specific noradrenergic reuptake inhibitor; maprotiline, a tetracyclic antidepressant which strongly inhibits the reuptake of noradrenaline but lacks inhibition of serotonergic reuptake; and fluoxetine, a selective serotonin reuptake inhibitor (SSRIs), (Yalcin, I., Belzung, C., and Surget, A., 2008). This model has since been repeated and reproduced several times and has been found out to be one of the best validated models of depression so far (Farooq, R. K., Isingrini, E., Tanti, A. *et al*, 2012).

UCMS is able to fulfill most of the nine criteria proposed by Belzung and Lemoine. It has been reproduced over and over again using BALB/c strain of mice, which makes it the species and strain specific, also validating the ontopathogenic reasons of exploiting the genetic predispositions of a certain strain. The triggering mechanism is the exposure to randomized mild stressors over a prolonged period of time. It leads to behavioral as well as biochemical alterations observed in clinically depressed subjects like helplessness, lack of self-care and inability to feel pleasure in day to day life. Regarding induction and remission criteria, UCMS is validated as the syndrome of behavioral despair and biological alterations is induced by stress and reversed by chronic treatment by antidepressants. Theoretical mechanisms underlying mood and biochemical alterations in humans are the same postulated in rodents although their exactness is yet to be confirmed in both humans and mice.

The general protocol of this model of stress consists of exposure of a vulnerable strain of mice to various stressors with another group of mice kept in standard housing conditions. The commonly employed stressors include wet bedding, frequent changes of saw dust, housing without saw dust, social stress, putting rat droppings in mice cages, light and dark alterations, predator sounds, contention stress and tilting of cages at 45 degrees. The stressors are initiated from milder to mild and moderate in intensity and are randomized so that they remain unpredictable to the animals. Two weeks after the initiation of the stress protocol, mice are started with antidepressant therapy, Fluoxetine intraperitoneally. Weekly coat state is recorded as an evolutionary measure of the effects of stress and AD treatment. It is a pharmacologically validated index in the said strain of mice. A combined stress and AD treatment is continued for as long as the effects of stress and AD treatment become obvious. At this moment, a battery of behavioral tests is applied according to the need of the protocol and the question posed.

Neuroinflammation and depression

Activation of microglia is indispensable in cases of brain injury in order to contain the injurious stimulus, via phagocytosis (Krysko, D. V., Vanden Berghe, T., D'Herde, K. *et al*, 2008). It has been suspected that immune system partakes in pathological process during the course of cleaning and protecting brain tissue from injury. Involvement of peripheral immune system activation following injury has been suspected to induce pathological process in the brain. The immune mechanisms native to brain itself have also been discovered. The unique immune system of the brain, although segregated from the prime immune system of the body by blood brain barrier, responds to immune alterations/reactions occurring in the periphery as well as , by immune as well as behavioral changes in the subject (Alexopoulos, G. S. and Morimoto, S. S., 2011). Clinical and preclinical studies are increasingly showing convincing results about mood disorders and their immune components. On one hand immune parameters are found to be activated in depressive episodes. On the other hand, immunotherapy has been found to induce symptoms which are very close to depressive illness itself. These markers include cytokines in brain and plasma.

Episodes of depression have been characterized by an increase in levels of various markers of inflammation, both centrally and peripherally. Acute stress has been associated with an increase in the levels of interleukin-1 β in rats (Nguyen, K. T., Deak, T., Owens, S. M. *et al*, 1998a). Acutely depressed and previously unmedicated patients were found to have higher concentration of pro-inflammatory cytokine IL-1β in cerebrospinal fluid (CSF) (Levine, J., Barak, Y., Chengappa, K. N. *et al*, 1999). Social stress in humans has been found to have triggered an increase in the circulating levels of IL-6, eventually resulting in activation of hypothalamo-pituitary (HPA) axis and its metabolic consequences like diabetes mellitus and coronary heart disease (Yudkin, J. S., Yajnik, C. S., Mohamed-Ali, V. *et al*, 1999). Major depression has also been associated with an increase in IL-6 levels in the blood (Papanicolaou, D. A., Wilder, R. L., Manolagas, S. C. *et al*, 1998b). IL-6 has been proposed to play a central role in the systemic consequences of psychological stress, mediating with stress through HPA axis and catecholamines leading to insulin resistance, coagulation abnormalities and endothelial dysfunction (Yudkin, J. S., Kumari, M., Humphries, S. E. *et al*, 2000b). Levels of IL-6 are found to be increased with age (Ershler, W. B. and Keller, E. T., 2000) and pregnancy and labor (Opsjln, S. L., Wathen, N. C., Tingulstad, S. *et al*, 1993). It caused an increase in fatty lesions in arteries in a mouse model providing a possible link between psychological stress and vascular diseases (Huber, S. A., Sakkinen, P., Conze, D. *et al*, 1999). Chronic unpredictable stress was noted to induce decrease in locomotor activity (depressive like behavior) as well as favor atherosclerosis through activating many markers of inflammation like C-Reactive proteins (CRP), IL-6 and elevated the concentration of VCAM-1 and ICAM-1 in plaques and plasma of apolipoprotein knockout mice (ApoE-

/-) (Zhang, T., Chen, Y., Liu, H. *et al*, 2010). Positive acute phase proteins were found to be raised while negative acute phase proteins decreased in an episode of depression, implying the fact that depression does initiate an inflammatory response in the body (Song, C., Dinan, T., and Leonard, B. E., 1994). Recent evidence suggests that sleep disturbances which are hallmark of depressive illness also cause activation of immune cells inside the brain leading to neuroinflammation and other cognitive deficits (Zhu, B., Dong, Y., Xu, Z. *et al*, 2012).

2.1. Evidence and Implications

As discussed earlier on, major depression is a recurrent illness, and frequency of episodes increases with each episode as well as with advancing age (Kasper, S., 1993). Increased incidence of depressive illness has been reported to be associated with advancing age, especially among those in nursing homes (Datto, C. J., Oslin, D. W., Streim, J. E. *et al*, 2002). Depression is also a frequent finding among the elderly (Palsson, S. P., Ostling, S., and Skoog, I., 2001), especially those with chronic medical illness like coronary heart disease and diabetes (Kinder, L. S., Kamarck, T. W., Baum, A. *et al*, 2002), Parkinson's disease (Edwards, E., Kitt, C., Oliver, E. *et al*, 2002), stroke (Whyte, E. M. and Mulsant, B. H., 2002) and cancer (Stommel, M., Given, B. A., and Given, C. W., 2002). Depression among the older age group has been significantly associated with vascular risk factors and is now being proposed that this form of illness be recognized as a separate entity as vascular depression (Alexopoulos, G. S., Meyers, B. S., Young, R. C. *et al*, 1997a;Alexopoulos, G. S., Meyers, B. S., Young, R. C. *et al*, 1997b) or geriatric depression (Camus, V., Kraehenbuhl, H., Preisig, M., Bula, C. J., and Waeber, G., 2004b) so that proper guidelines can be formulated. From this data, we can infer that depression is associated with aging, medical illness and chronic stress. The pathophysiology of depression based on previously reviewed data suggests that cytokine–serotonin interaction through the activation of kynurenine pathway results in tryptophan depletion (Miller, A. H., Maletic, V., and Raison, C. L., 2009). Similar mechanism has been postulated to explain the emergence of depressive symptoms following stroke (Spalletta, G., Bossu, P., Ciaramella, A. *et al*, 2006) as well as interferon-α induced depression (Wichers, M. C. and Maes, M., 2004) both of which have the commonality of inflammatory components activation for extended durations. All of the above mentioned risk factors of depression including aging, inflammatory illnesses as well as vascular disorders are characterized by increased levels of inflammatory cytokines and can be implicated in tryptophan depletion. Together, this may implicate the persistence of pro-inflammatory state in giving rise to affective symptoms and may also lead to degenerative changes if left untreated. Epidemiological data also suggests that depressed subjects are vulnerable to develop degenerative diseases in the long run (Hemmerle, A. M., Herman, J. P., and Seroogy, K. B., 2012).

The pathophysiology of depression based on previously reviewed data

suggests cytokine–serotonin interaction through the enzyme IDO which activates the kynurenine pathway and results in tryptophan depletion and subsequent depression (Miller, A. H., Maletic, V., and Raison, C. L., 2009). As explained in figure 7, the mechanism of tryptophan depletion under the influence of neuroinflammation involves increased enzymatic activity of IDO which accelerates the production of kynurenine (Kang, A., Hao, H., Zheng, X. *et al*, 2011). A recent report noted that IDO activity at baseline and follow up was associated with depressive symptoms in women (Elovainio, M., Hurme, M., Jokela, M. *et al*, 2012). Although initially activated to perform a protective function, the IDO goes on to become a cause of induction of depressive symptoms and degenerative illnesses in due course of time (Kwidzinski, E. and Bechmann, I., 2007). Homeostatic mechanisms meant to keep a balance between pro and anti-inflammatory forces are disrupted in disease states, whether sickness behavior or else (for a review see (Schiepers, O. J., Wichers, M. C., and Maes, M., 2005a). Disease progresses when balance between pro-inflammatory cytokines and anti-inflammatory cytokines, pro-neurodegeneration compound quinolinate and neuroprotective kynurenate fails to maintain itself (Myint, A. M. and Kim, Y. K., 2003).

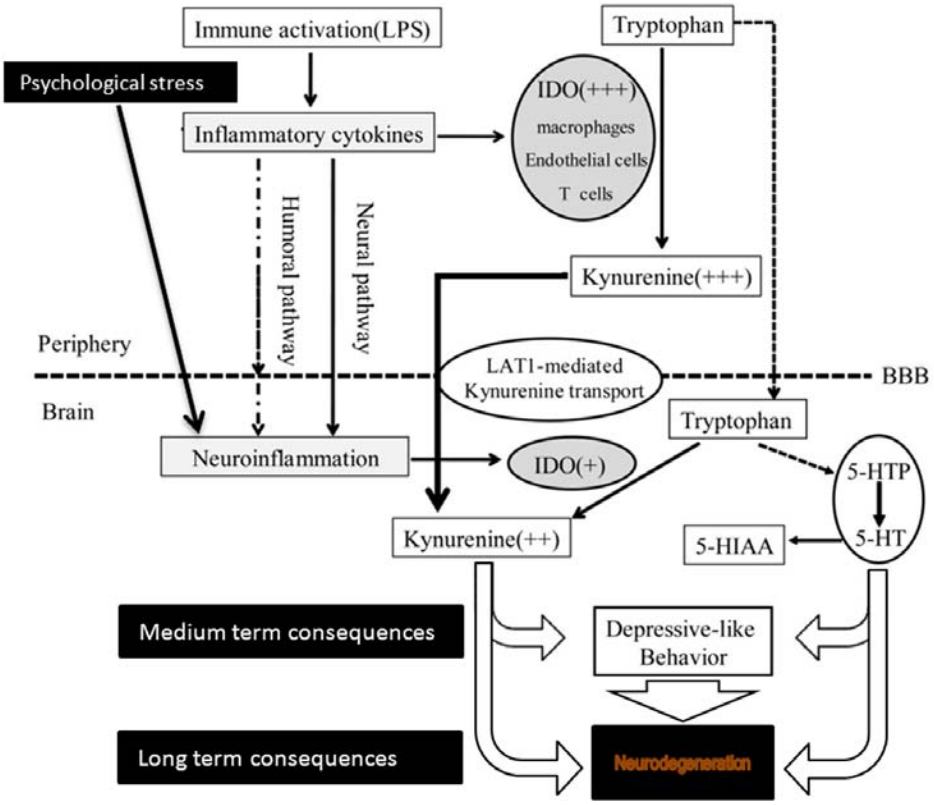

Figure 7: Neurodegeneration hypothesis of depression. Figure adopted from (Kang, A., Hao, H., Zheng, X., Liang, Y., Xie, Y., Xie, T., Dai, C., Zhao, Q., Wu, X., Xie, L., and Wang, G., 2011)

2.2. Components of Neuroinflammatory process

2.2.1. Immune cells of brain: Microglia

Brain comprises mainly two types of cells, neurons and their supporting cells called glia. The term glia includes three types of cells namely astrocytes, oligodendrocytes and microglial cells, of which, microglial population is the most important one when we consider the immune reaction of brain to an injurious stimulus (Monif, M., Burnstock, G., and Williams, D. A., 2010). They originate from the mainstream immune lineage, the monocyte macrophage cells which migrate into the brain at early stages of development and go through several morphological changes before assuming the role of ready-to-go microglial cells (Kaur, C., Hao, A. J., Wu, C. H. *et al*, 2001). Microglia are found throughout the brain and make 10-12% of total brain cells. Their density ranges from 0.5-16.5% in different structures of the brain; hippocampus, olfactory telencephalon, basal ganglia and substantia nigra being the most populated brain structures (Block, M. L., Zecca, L., and Hong, J. S., 2007). They act as first line of defense in virtually any stimulus altering the balance of immunity inside CNS, from within as well as from without (Lemstra, A. W., Groen in't Woud, J. C., Hoozemans, J. J. *et al*, 2007). In so called resting state characterized by a ramified structure and low expression of immunological molecules, microglia continuously patrol their microenvironment, keeping themselves aware of their surroundings which make them capable of responding extremely quickly in case of recognition of an injurious stimulus in their vicinity (Davalos, D., Grutzendler, J., Yang, G. *et al*, 2005;Nimmerjahn, A., Kirchhoff, F., and Helmchen, F., 2005). The components of this microglial warning system are membrane molecules called "Pattern Recognition Receptors" or PRRs (Akira, S., Uematsu, S., and Takeuchi, O., 2006) which include various Toll-like receptors (TLRs), scavenger receptors and the complement receptor 3 Mac 1 (Olson, J. K. and Miller, S. D., 2004;Downes, C. E. and Crack, P. J., 2010;Husemann, J., Loike, J. D., Anankov, R. *et al*, 2002;Ross, G. D., 2000) sometimes also called the danger-associated molecular patterns (DAMPs) or pathogen associated molecular patterns (PAMPs). TLRs are type I membrane proteins characterized by an ectodomain composed of leucine rich repeats (LRR) that are responsible for recognition of PAMPs and a cytoplasmic domain homologous to the cytoplasmic region of the IL-1 receptor, known as the TIR domain, which is required for downstream signaling. They play central role in the induction of innate immune responses and consequently the eventual development of adaptive immune responses (Akira, S., Uematsu, S., and Takeuchi, O., 2006). Based on their specificity for various molecules, TLRs have been divided into various types and make as much as 11 and 13 different types in humans and in mice respectively, each type capable of recognizing a specific PAMP (Akira, S., Uematsu, S., and Takeuchi, O., 2006). TLR4 among others is the best characterized one which plays a critical role in LPS induced phagocytic activity of immune cells (Wu, T. T., Chen, T. L., and

Chen, R. M., 2009). The cytoplasmic portion of TLRs is similar to that of the interleukin (IL)-1 receptor family, and is now called the Toll/IL-1 receptor (TIR) domain. TIR domain initiates the activation of TLR signaling pathway which further consists of a MyD88-dependent (common to all TLRs) and a MyD88-independent pathway (specific for TLR3 and TLR4) (Akira, S., Takeda, K., and Kaisho, T., 2001;Li, C., Zienkiewicz, J., and Hawiger, J., 2005). LPS induced inflammatory reaction uses MyD88-dependant pathway as a MyD88 knockout strain of mice did not show an inflammatory response to LPS (Kawai, T., Adachi, O., Ogawa, T. *et al*, 1999) neither did it produce IL-6 in response to TLR-5 ligand flagellin (Hayashi, F., Smith, K. D., Ozinsky, A. *et al*, 2001). Consequences of stimulation of TLR3 and TLR4 include cleavage of inactive pro-IL-1 β into its mature and active form, the IL-1 β by stimulation of caspase-1 (Martinon, F. and Tschopp, J., 2007;Maelfait, J., Vercammen, E., Janssens, S. *et al*, 2008;Li, P., Allen, H., Banerjee, S. *et al*, 1995). Caspase-1 deficiency mutation as well as its specific inhibitor the tetrapeptide YVAD (Tyr-Val-Ala-Asp) both results in a failure of IL-1β maturation (Sanz, J. M. and Di, Virgilio F., 2000;Perregaux, D. G. and Gabel, C. A., 1998). However, in the absence of a secondary stimulus, almost 95% of pro-IL β is not converted to its active form. The post-translational maturation of IL-1β has been discussed further in the next section.

The other aspects of injury induced microglial activation and subsequent events involve adenosine triphosphate (ATP). Whatever the cause of injury is; it evokes liberation of various neurotransmitters, cytokines and chemokines from dying or injured cells. One such signaling molecule is ATP which has been found to be present at and around the site of injury in high quantities (Pellegatti, P., Raffaghello, L., Bianchi, G. *et al*, 2008;Idzko, M., Hammad, H., van, Nimwegen M. *et al*, 2007;Wang, X., Arcuino, G., Takano, T. *et al*, 2004) and acts like an informer/chemotactic factor for microglia, in response to which microglia go through a series of morphological changes, taking the role of macrophages (Davalos, D., Grutzendler, J., Yang, G., Kim, J. V., Zuo, Y., Jung, S., Littman, D. R., Dustin, M. L., and Gan, W. B., 2005). ATP also plays its role in the post-translational maturation and release of IL-1 β *in vitro* (Perregaux, D. and Gabel, C. A., 1994a). It also promotes cell survival in cases of a low dose inflammatory challenge by a TLR4 ligand bacterial antigen LPS whereas higher doses of the same exert the opposite effect (Harada, K., Hide, I., Seki, T. *et al*, 2011). High doses of ATP can cause cell death in absence of a traumatic injury, an effect which is directly proportional to the activity of P2X7 receptors in that tissue (Wang, X., Arcuino, G., Takano, T., Lin, J., Peng, W. G., Wan, P., Li, P., Xu, Q., Liu, Q. S., Goldman, S. A., and Nedergaard, M., 2004). The extent of this reaction, however, is subject to extent of the stimulus inducing its release, and indirectly, amount of ATP released by the injured tissue.

The primary purpose of microglial activation is to contain the injurious stimulus and subsequently remove the toxic agents as well as cellular

debris in order to minimize the eventual damage. They infiltrate site of injury by following the so called 'find-me' signals secreted from the injured or dying cells (Ravichandran, K. S., 2011). As a result of injury, a protein called phosphatidylserine (PS) is exteriorized which is interpreted by microglia as an 'eat me' signal (Fadok, V. A., Bratton, D. L., Rose, D. M. *et al*, 2000), although 'eat-me' signal includes other components in addition to PS (Koizumi, S., Shigemoto-Mogami, Y., Nasu-Tada, K. *et al*, 2007). Microglia, before phagocytizing the critically injured cells, also make sure that they lack 'don't eat me' signal as well (Lauber, K., Blumenthal, S. G., Waibel, M. *et al*, 2004) after which they are eaten up. Evidence suggests that PS expression by the injured cells is also secondary to the presence of microglia (Neher, J. J., Neniskyte, U., Zhao, J. W. *et al*, 2011a) which makes their role even more significant. The PS exposure, the so called 'eat me' signal, can be reversibly stimulated by LPS or lipoteichoic acid (LTA) through activation of TLR2/4 which can either lead to phagocytosis or a return to healthy state in case phagocytosis is blocked (Neher, J. J., Neniskyte, U., Zhao, J. W. *et al*, 2011b). This means that LPS induced expression of TLR2/4 signals may cause microglia to eat the otherwise healthy cells too. In the ideal case though, when the battle is over, microglia begin the process of healing and repair by inviting stem cells to the site of injury (Aarum, J., Sandberg, K., Haeberlein, S. L. *et al*, 2003), favoring the remaining neurons to make new synapsis (Bessis, A., Bechade, C., Bernard, D. *et al*, 2007) as well as secretion of neurotrophic factors to promote cell survival (Kreutzberg, G. W., 1996;Alexopoulos, G. S. and Morimoto, S. S., 2011) assuming their noble work of repair and construction again by releasing growth factors. They include nerve growth factor (NGF), brain derived neurotrophic factor (BDNF), basic fibroblast growth factor (BFGF), glial cell-line derived neurotrophic factor (GDNF0, insulin like growth factor (IGF), hepatocyte growth factor (HGF), Neurotrophins (NTs) as well as platelet derived growth factor (PDGF); (Monif, M., Burnstock, G., and Williams, D. A., 2010;Yoshihisa Kitamura, Daijiro Yanagisawa, Kazuyuki Takata *et al*, 2009).

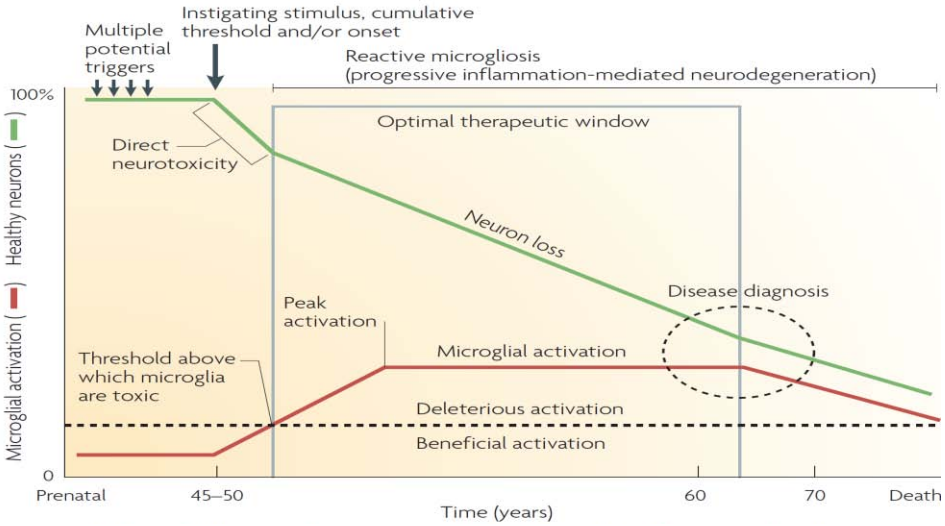

Figure 8: Microglial activation and its neurodegenerative consequences in therapeutic

relevance: Adapted from Microglia-mediated neurotoxicity: uncovering the molecular mechanisms by Block et al 2007.

The extent of inflammatory action of microglia is controlled by a poorly understood/hypothetical communication between injured neurons and microglia, disruption of which leads to excesses in microglial inflammatory response and its deleterious consequences (Polazzi, E. and Contestabile, A., 2002). Microgliosis or reactive microgliosis is the term applied to excessive accumulation of microglia at certain sites in the brain which eventually shifts the balance of microglial activity in favor of destruction (Block, M. L., Zecca, L., and Hong, J. S., 2007). As shown in figure 9, rapid neuronal loss when excessive microglial activation continues unabated and leads to disease symptoms manifestations once significant injury has occurred. From the initiation of the microglial activation process to appearance of symptoms, an important therapeutic window presents itself which can be benefitted from only if risk factors are identified and diagnosis is sought early on.

In addition to non-viable neuronal cells, activated microglia engulf the stressed but still viable cells as well, activation being the cause of their impairment to differentiate between a apoptotic and a potentially non-apoptotic cell (McArthur, S., Cristante, E., Paterno, M. *et al*, 2010). This puts a question mark on the assumption that phagocytosis is limited to dead cell populations only (Neher, J. J., Neniskyte, U., and Brown, G. C., 2012). This phagocytic behavior is being postulated to be cause rather than being consequence of neuronal loss and has also given birth to the term "primary phagocytosis" or phagoptosis" (Brown, G. C. and Neher, J. J., 2012).

Microglias in this process take a devastating role. The burden of such structural and functional alterations is borne by hippocampus, one of the areas where density of microglia is the highest, resulting in impairment of neurogenesis and behavioral manifestations (Kempermann, G. and Neumann, H., 2003). These inflammation-mediated behavioral consequences overlap with depressive symptoms to a significant degree which has earned them the term of "sickness behavior".

Sickness behavior is one of the outcome consequences of an acute infection or an acute exacerbation of a chronic inflammatory illness or an inflammatory challenge. This behavior is homeostatic and protective in nature, which by altering the appetite, temperature, social activity level of the organism conserves body's energy for metabolic challenges (Kluger, M. J., 1991). This syndrome of behavioral deficit is very similar to symptoms of depression. It is a set of neurobehavioral and neurophysiological symptoms which include weakness, malaise, reduced food and fluid intake, lethargy and lack of motivation etc. most of which overlap with those of the major depressive disorder (Dantzer, R., 2001;Raedler, T. J., 2011). The

psychological and behavioral components of sickness behavior represent, together with fever response and associated neuroendocrine changes, a highly organized strategy of the organism to fight infection (Dantzer, R., O'Connor, J. C., Freund, G. G. *et al*, 2008).

How does microglial activation translate into a change in subject's behavior? Microglial proliferation in prefrontal cortical areas is a consequence of stress which alters subject's response to stress and leads to sickness behavior, possibly by modulating neuronal activity and connectivity (Graeber, M. B., 2010;Wake, H., Moorhouse, A. J., Jinno, S. *et al*, 2009). Secondly, Hinwood *et al.* showed that stress induced microglial proliferation influenced neuronal activity in the prefrontal cortex resulting in impairment of working memory and performance. They also showed that an inhibitor of microglial activation also resulted in reinstatement of the deranged cortical functions (Hinwood, M., Morandini, J., Day, T. A. *et al*, 2012), which explains the potential mechanism behind behavioral consequences of microglial activation. In addition to this, the answer to this question can also be traced by studying the mechanism by which microglia interact with other determinants of an animal's behavior like neurogenesis and negative feedback system of HPA axis.

Microglia do contribute to adult neurogenesis in more than one ways, increasing neurogenesis in sub-granular zone of the dentate gyrus in mice and secreting anti-inflammatory pro-neurogenic cytokine TGF β thus helping the new neurons integrate into the neuronal networks although this gentle attitude is limited to a ramified state of microglia and not to a phagocytic state (Battista, D., Ferrari, C. C., Gage, F. H. *et al*, 2006). Chronic microglial activation/neuroinflammation impairs this process (the functional integration of adult born neurons) disrupting the behavioral and biochemical outcomes of the process of neurogenesis (Belarbi, K., Arellano, C., Ferguson, R. *et al*, 2012). It is yet to be seen what determines between the eventual detrimental or beneficial roles of microglial activation, whether there is a different phenotype for each one of them, or whether it is the type of PRRs activated (Gomes-Leal, W., 2012) or even, the extent and chronicity of their activation. With the advent of phagocytic state and shifting of balance in favor of pro-inflammatory situation, an anti-neurogenic as well as behavioral deficit effect becomes inevitable. This is further discussed in the following section.

2.2.2. Cytokines

The cytokines are a diverse group of proteins that may be considered as the hormones of the immune system. They are low molecular weight (<200 amino acids) proteins and include interleukins (IL-1 To IL-24), tumor necrosis factors (TNF) and transforming growth factors (TGF β 1-3) (Wilson, C. J., Finch, C. E., and Cohen, H. J., 2002). They make an important component of immune response. Inside the brain they are mediators of inflammatory reaction of microglia. They are released by the cells of

immune origin, predominantly microglia and are divided into two categories based upon their contribution to the process of inflammation, pro-inflammatory and anti-inflammatory cytokines. Pro-inflammatory cytokines which include IL-1β, TNF and IL-6 while anti-inflammatory cytokines which include IL-10 and others. In homeostatic conditions a balance is maintained between pro-inflammatory and anti-inflammatory cytokines which is quite understandably disrupted in pathological conditions. In disease conditions like in response to an immune challenge as in case of TLR4 ligand bacterial endotoxin lipopolysaccharide (LPS), TNF alpha is released from microglia which stimulates other notable pro-inflammatory cytokines like IL-1 β and IL-6 (Qin, L., He, J., Hanes, R. N. *et al*, 2008). Cytokines affect the brain homeostasis from within as well as from without. From periphery, they are believed to affect the brain immune system by seeping through circumventricular organs due to weak blood brain barrier or through active transport (Banks, W. A., Kastin, A. J., and Broadwell, R. D., 1995). Alternatively, it can give rise to relatively small sized prostaglandins which can readily cross blood brain barrier and convey the inflammatory message (Watkins, L. R., Maier, S. F., and Goehler, L. E., 1995). Certain cytokines can pass through blood brain barrier in physiological states (Banks, W. A., 2005) while neuroinflammatory conditions (de Vries, H. E., Kuiper, J., de Boer, A. G. *et al*, 1997) as well as stress (Lytinas, M., Kempuraj, D., Huang, M. *et al*, 2003) can potentially modulate BBB permeability through a regulatory function by corticotropin releasing hormone and mast cells (Esposito, P., Chandler, N., Kandere, K. *et al*, 2002). Several other pro-inflammatory mediators affect permeability of BBB in various conditions, for a review see (Abbott, N. J., 2000). Cytokines, whether originated in the periphery or secreted from microglia as a part of their inflammatory reactivity, take part in inflammatory cascade and consequent injury repair function, assuming central role in an individual's behavioral response to the inflammatory stimulus (Godbout, J. P., Chen, J., Abraham, J. *et al*, 2005;Combrinck, M. I., Perry, V. H., and Cunningham, C., 2002).

Role of cytokines in psychiatric disorders, particularly in mood disorders, has been highlighted by several studies, both clinical and preclinical. Clinical studies have consistently shown that increased levels of pro-inflammatory cytokines to be related to depressive illness (Papanicolaou, D. A., Wilder, R. L., Manolagas, S. C., and Chrousos, G. P., 1998a;Schiepers, O. J., Wichers, M. C., and Maes, M., 2005b;Yirmiya, R., Pollak, Y., Morag, M. *et al*, 2000a;Tuglu, C., Kara, S. H., Caliyurt, O. *et al*, 2003;Lanquillon, S., Krieg, J. C., Bening-Abu-Shach, U. *et al*, 2000b;Maes, M., Bosmans, E., and Meltzer, H. Y., 1995;Mikova, O., Yakimova, R., Bosmans, E. *et al*, 2001;Sluzewska, A., Rybakowski, J. K., Laciak, M. *et al*, 1995). As shown in figure 10, peripheral and central inflammatory components are prone to be stimulated by a variety of disease conditions including stress, infections and trauma, alone as well as in combinations, which results in neurochemical alterations and

appearance of behavioral symptoms. Preclinical studies have reported elevated concentration of pro-inflammatory as well as anti-inflammatory cytokines in various models of depression (reviewed in (Song, C. and Wang, H., 2011;Zunszain, P. A., Anacker, C., Cattaneo, A. *et al*, 2011) . Pro-inflammatory cytokine IL-1β has been found to be increased and related to antidepressant response in depressed subject (Rethorst, C. D., Toups, M. S., Greer, T. L. *et al*, 2012) particularly in elder subjects suffering from depression, directly proportional to the severity of illness (Thomas, A. J., Davis, S., Morris, C. *et al*, 2005).

Elevated cytokines may be important in depression for several reasons. One of the mechanisms by which cytokines contribute to mood disorders is their effect on hippocampal neurogenesis, which is an important part of pathophysiology and also basis for pharmacotherapy of major depression (Saarelainen, T., Hendolin, P., Lucas, G. *et al*, 2003;Nibuya, M., Morinobu, S., and Duman, R. S., 1995). Neurogenesis is the target of intervention which is achieved by promotion of brain derived neurotrophic factor (BDNF) expression in hippocampus brought about by selective serotonin reuptake inhibitor, the group of agents which have been mainstay of depression pharmacotherapy till now. Increased BDNF favors neurogenesis by promoting cell proliferation and survival of neural progenitor cells (Malberg, J. E., Eisch, A. J., Nestler, E. J. *et al*, 2000;Nibuya, M., Morinobu, S., and Duman, R. S., 1995;Sairanen, M., Lucas, G., Ernfors, P. *et al*, 2005;Lee, J., Duan, W., and Mattson, M. P., 2002;Brunoni, A. R., Lopes, M., and Fregni, F., 2008). Inflammatory activity has been found to reverse it, decreasing BDNF, resulting in decreased cellular proliferation and survival (Ekdahl, C. T., Claasen, J. H., Bonde, S. *et al*, 2003b). The role of cytokines in mood disorders is further strengthened by the demonstration that pro-inflammatory cytokines are able to activate the hypothalamus–pituitary–adrenal (HPA) axis (Crane, J. W., Buller, K. M., and Day, T. A., 2003).

Anti-inflammatory cytokines, on the other hand, modulates the initiation of depressive like behavior in experimental animals. Interleukin-10 knockout mice showed decreased latency to immobility in a forced swim test where administration of IL-10 was able to reverse behavior of helplessness (Mesquita, A. R., Correia-Neves, M., Roque, S. *et al*, 2008a). IL-10 receptor1 is located on rat microglia which responds to inflammatory stimuli such as LPS injection (Ledeboer, A., Breve, J. J., Wierinckx, A. *et al*, 2002). It inhibits pro-inflammatory cytokine production by Glial cells stimulated by LPS in a dose dependent manner (Ledeboer, A., Breve, J. J., Poole, S. *et al*, 2000).

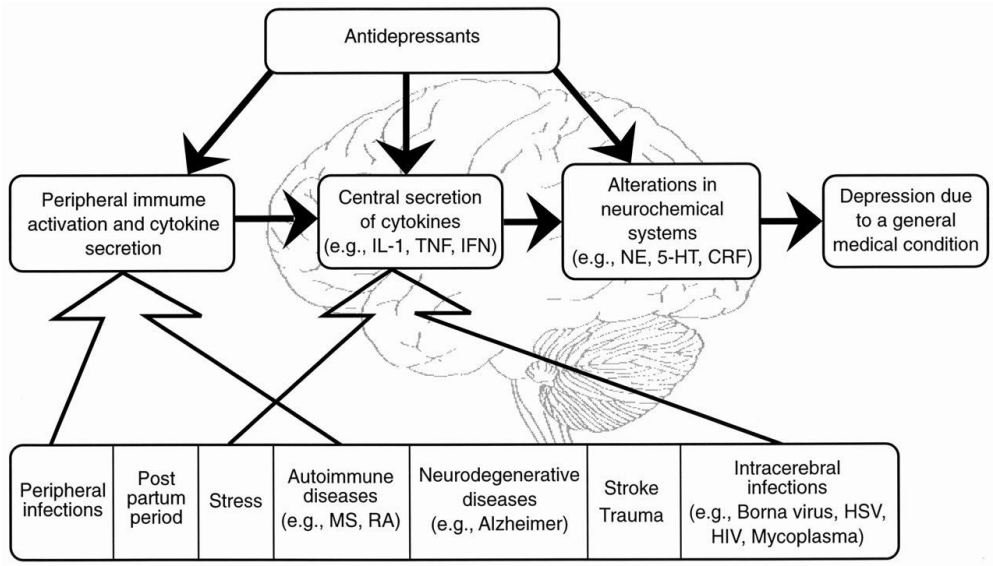

Figure 9: Disease state and cytokine alterations in causing depression (Yirmiya, R., Pollak, Y., Morag, M. *et al*, 2000b).

Another mechanism relating pro-inflammatory cytokines to mood is their capacity to alter metabolism of serotonin. Levels of serotonin have been found to be altered in animal models exposed to proinflammatory cytokines (Dunn, A. J., Wang, J., and Ando, T., 1999). One of the possible mechanism by which they evoke metabolic alterations is to induce the indoleamine-2,3-dioxygenase (IDO) enzyme, which catalyzes the rate-limiting step in the synthesis of kynurenine from dietary tryptophan (Laugeray, A., Launay, J. M., Callebert, J., Surget, A., Belzung, C., and Barone, P. R., 2010;Schrocksnadel, K., Wirleitner, B., Winkler, C. *et al*, 2006;Heyes, M. P., Saito, K., and Markey, S. P., 1992a;Byrne, G. I., Lehmann, L. K., Kirschbaum, J. G. *et al*, 1986). Proinflammatory cytokines, including IFN-gamma, IL-6 and TNF-alpha have been shown to increase the expression of IDO in immune cells both central and peripheral (Connor, T. J., Starr, N., O'Sullivan, J. B. *et al*, 2008;Tu, H., Rady, P. L., Juelich, T. *et al*, 2005). Thus, activation of these cell types can increase metabolism of tryptophan, which depletes the availability of precursor of serotonin and melatonin which affects mood (Heyes, M. P., Saito, K., and Markey, S. P., 1992b;Mellor, A. L. and Munn, D. H., 1999). In addition, and more importantly, kynurenine metabolite quinolinic acid which is an endogenous *N*-methyl-D-aspartate (NMDA) agonist can alter neurotransmission along glutamatergic pathways (Stone, T. W. and Perkins, M. N., 1981;Wichers, M. C., Koek, G. H., Robaeys, G. *et al*, 2005). As a potent NMDA receptor agonist, quinolinic acid may lead to hippocampal neuron damage and apoptosis (Stone, T. W. and Behan, W. M., 2007;Schwarcz, R., Whetsell, W. O., Jr., and Mangano, R. M., 1983). Lastly, elevated levels of cytokines may also deplete serotonin by reducing tryptophan availability as a consequence of reduced food intake as a part of sickness behavior since dietary intake can interfere with TRP levels (Plata-Salaman, C. R., 1998;Reichenberg, A., Yirmiya, R., Schuld, A. *et al*, 2001;Smith, K. A., Fairburn, C. G., and Cowen, P. J., 1997). These mechanisms may contribute to atrophy of hippocampus as well as to the

symptoms of major depression. The clinical significance of the kynurenine pathway is based on studies which state increased concentrations of kynurenine and its metabolites in depressed patients (Myint, A. M., Kim, Y. K., Verkerk, R. *et al*, 2007;Moller, S. E., Kirk, L., and Honore, P., 1982;Wood, K., Harwood, J., and Coppen, A., 1978) and report correlation between concentration of these metabolites with symptoms aggravation and remission following treatment (Mackay, G. M., Forrest, C. M., Christofides, J. *et al*, 2009).

Among all the Proinflammatory cytokines, IL-1 β has been found to be the most important one in translating the effect of stress onto respective physiological and behavioral processes. The role of IL-1 β has been discussed in detail as follows.

2.2.2.1. *IL*-1 β

Although a host of pro-inflammatory cytokines take part in mediating their effects inside the brain, IL-1 β was the first one to be implicated in the regulation of neuroendocrine response to stress, the so called hypothalamic pituitary-adrenal axis (HPA) (Berkenbosch, F., van, Oers J., del, Rey A. *et al*, 1987;Bernton, E. W., Beach, J. E., Holaday, J. W. *et al*, 1987;Sapolsky, R., Rivier, C., Yamamoto, G. *et al*, 1987a). IL-1 family consists of proteins encoded by a variety of genes which include IL-1 alpha, IL-1 β, IL receptor (IL-1R) antagonist (IL-1RA) and two IL-1 receptor accessory proteins (IL-1R AcP I and II) (Vitkovic, L., Bockaert, J., and Jacque, C., 2000). Among them IL-1 β is the most studied pro-inflammatory cytokines to date. It is produced predominantly by cells of immune origin; macrophages in the periphery and microglia inside the brain (Giulian, D., Baker, T. J., Shih, L. C. *et al*, 1986;Dinarello, C. A., 1996a). As microglia are known to be the champions of all types of brain immune eventualities, IL-1 β is considered to be a part of virtually every inflammatory event inside brain, be it ischemia, seizures, mechanical injury, fever, infection, multiple sclerosis, AIDS, Down's syndrome or Alzheimer's disease (reviewed in (Vitkovic, L., Bockaert, J., and Jacque, C., 2000) and (Stock, C., Schilling, T., Schwab, A. *et al*, 2006)). During such activity periods, IL-1 gene expression as well as protein production increases which in addition to exerting its own proper inflammatory effects, also enhances production of other pro-inflammatory cytokines (such as IL-6) as well as increases development of T cell clones (Dinarello, C. A., 1996a). The release in an inactive form followed by activation is a complex process discussed as under.

Post-translational Processing: As discussed in the previous section, inflammatory message of a peripheral origin can reach the brain even from periphery. Once it arrives inside the brain, it is translated into recruitment of immune cells and subsequent increase in concentration of inflammatory cytokines (predominantly IL-1 β) centrally. IL-1 β is released from microglia in response to/as a consequence of activation of toll-like receptors on T cells membrane.

LPS is a principal activator of TLR signaling which activates MAPK (mutagen-activated protein kinase0 and/or NF-κB 9 nuclear factor kappa-light-chain-enhancer of activated B cells) cascades, favoring the activity of the IL-1β promoter resulting in the formation pro-IL-1β (molecular mass 31kDa) (Kawai, T. and Akira, S., 2007). This pro-IL-1β is slowly secreted into cytosol and needs to be cleaved into mature IL-1β (molecular mass 17-kDa) by caspase-1 (as discussed in the previous section (*Immune cells of Brain: microglia*), which is itself cleaved from pro-caspase-1 by caspase recruiting domain (ASC). This caspase recruiting domain is a part of an apoptosis-associated speck-like protein which originates from stimulation of cytoplasmic receptors like nucleotide binding domain (NOD)-like receptors by a secondary stimulus such as adenosine triphosphate (ATP) or danger associated molecular patterns (DAMP)-inflammosome complex (Petrilli, V., Dostert, C., Muruve, D. A. *et al*, 2007). In absence of this secondary stimulus (ATP), amount of IL-1β is insufficient to make an impact supposedly due to an indispensable ATP dependent post-translational processing (Griffiths, R. J., Stam, E. J., Downs, J. T. *et al*, 1995). Studies using different ligands like ATP (Ferrari, D., Chiozzi, P., Falzoni, S. *et al*, 1997c;Sanz, J. M. and Di, Virgilio F., 2000), LPS (Yao, J., Keri, J. E., Taffs, R. E. *et al*, 1992;Ferrari, D., Chiozzi, P., Falzoni, S., Hanau, S., and Di, Virgilio F., 1997c), ADP or AMP (Chakfe, Y., Seguin, R., Antel, J. P. *et al*, 2002) have shown that IL-1β release from microglia in its pro form and its subsequent conversion to its active form involves a process of post-translational processing and is mediated by $P2X_7$ receptor (187-189).

Several other members of pro-inflammatory protein family came into their active being through the same mechanism including IL-1α and IL-18 (Dinarello, C. A., 1996b;Reddy, P., 2004) and IL-1 receptor antagonist (IL-1Ra), (Arend, W. P. and Gabay, C., 2000) which binds to type I IL-1 receptor (IL-1RI) and blocks IL-1-dependent signal transduction, thus functioning as an endogenous, IL-1-selective inhibitor of inflammation (Dinarello, C. A., 2000). The mechanism of this post-translational processing of pro IL-1β to IL-1β is not fully understood, but is believed to involve the P2X7 (in detail in the following section). Release of IL-1β is proportional to the magnitude of the ensuing stimulus and so are its effects, both beneficial and detrimental (Li, Q., Luo, X., and Muallem, S., 2005).

Epidemiology: Elevated levels of IL-1β has been noted in chronic inflammatory diseases such as arthritis, scleroderma, systemic lupus erythematosus, vasculitis, sepsis, septic shock, and in the presence of atherosclerotic lesions leading to myocardial infarction (Dinarello, C. A., 2002). IL-1 β levels were found to be higher in women with symptoms of depression as compared to those who didn't have any such symptom one month postpartum (Corwin, E. J., Johnston, N., and Pugh, L., 2008) These statistics are, however, refuted by Ovaskainen et al (Ovaskainen, Y.,

Koponen, H., Jokelainen, J. *et al*, 2009) who observed that concentration of IL-1 β is not increased in an episode of depression. Rather they observed an increase in the level of IL-1 receptor antagonists (IL-1 β RA). Lowered levels of Zinc during an episode of depression has been proposed to originate from increased levels of IL-1 which results in sequestration of metallothionen, the zinc binding protein found in liver (Cousins, R. J. and Leinart, A. S., 1988).

Whether originated peripherally or released centrally after LPS challenge, it contributes to wide range of inflammatory and degenerative processes in the brain, including Alzheimer's disease, Parkinson's disease (Csolle, C. and Sperlagh, B., 2010). Moreover, it also exerts regulatory effects on physiological phenomena like feeding, sleep and HPA axis (Vitkovic, L., Bockaert, J., and Jacque, C., 2000). Apart from its pronounced role in known inflammatory processes, its effects on brain and behavior under physiological conditions are interesting too. It suppresses the feeding behavior in an animal model by acting directly on the central nervous system, along with tumor necrosis alpha (Plata-Salaman, C. R., Oomura, Y., and Kai, Y., 1988), an effect which is carried out by the up-regulation of IL-1R and can be blocked by administering its antagonist, IL-1RA (Kent, S., Bluthe, R. M., Dantzer, R. *et al*, 1992b).

IL-1 β and stress: IL-1 β is the one that has been implicated in majority of the stress related consequences in the brain. It is an important mediator of host response to infections undoubtedly through inflammation (Dinarello, C. A., 1996b;Dinarello, C. A., 2002). It has a bilateral relation with stress. Stress as well as depression cause an elevation in IL-1 β concentration (Nguyen, K. T., Deak, T., Owens, S. M., Kohno, T., Fleshner, M., Watkins, L. R., and Maier, S. F., 1998a;Shintani, F., Nakaki, T., Kanba, S. *et al*, 1995;Nguyen, K. T., Deak, T., Owens, S. M. *et al*, 1998b;Deak, T., Bordner, K. A., McElderry, N. K. *et al*, 2005;Johnson, J. D., Campisi, J., Sharkey, C. M. *et al*, 2005;Anisman, H., Ravindran, A. V., Griffiths, J. *et al*, 1999) and IL-1 β when injected or released from inflammatory cells as a response to stress or infection, exerts negative behavioral effects (sickness behavior) as well as hyperthermia (Kluger, M. J., Kozak, W., Conn, C. A. *et al*, 1998). Sickness behavior can also be induced by a peripheral immune challenge in form of bacterial antigen LPS which induces the release of IL-1 by macrophages and ramified microglial cells (Smith, R. S., 1991b;Buttini, M. and Boddeke, H., 1995;van Dam, A. M., Brouns, M., Louisse, S. *et al*, 1992). Inflammatory and behavioral consequences of lipopolysaccharide challenge can be effectively blocked using IL-1RA, the antagonist of IL-1 receptors (Marsh, C. B., Moore, S. A., Pope, H. A. *et al*, 1994;Abraham, J. and Johnson, R. W., 2009). Vagal afferent fibers have also been found to carry its message to the brain as vagotomy blocks many of central effects otherwise observed with peripheral immune activation including behavioral effects of

peripherally injected IL-1 β (Bluthe, R. M., Michaud, B., Kelley, K. W. *et al*, 1996;Gaykema, R. P., Goehler, L. E., Hansen, M. K. *et al*, 2000;Watkins, L. R., Maier, S. F., and Goehler, L. E., 1995). The negative effect on the exploratory behavior in mice was also attenuated in vagotomized animals injected with LPS peripherally although fever persisted (Luheshi, G. N., Bluthe, R. M., Rushforth, D. *et al*, 2000).

IL-1 β contributes to induction of depressive like behavior in many ways. It stimulates the adrenocortical axis at the level of brain through stimulation of release of corticotropin releasing factor (CRF) which is the principal mediator of most of the behavioral effects of stress (Sapolsky, R., Rivier, C., Yamamoto, G. *et al*, 1987b). In addition, IL-1 β also inhibits hippocampal long-term potentiation (Murray, C. A. and Lynch, M. A., 1998), down-regulates hippocampal brain derived neurotrophic factor (Barrientos, R. M., Sprunger, D. B., Campeau, S. *et al*, 2003) and impairs hippocampal-dependent contextual fear conditioning (Pugh, C. R., Nguyen, K. T., Gonyea, J. L. *et al*, 1999).

IL-1 β & HPA Axis: Specific effects of IL-1β on regulation of HPA axis are of great importance. HPA axis is undoubtedly the most important hormonal mechanism for stress response regulation. As stated in the HPA dysregulation hypothesis of depression pathophysiology, the negative feedback of glucocorticoid secretion is important to maintain homeostasis. Chronic stress in most of its forms acts to impair the negative feedback control of glucocorticoid secretion which leads to raised glucocorticoid levels beyond safe levels for prolonged periods of time and results in various effects on brain structure and function. These effects include modulation of adult neurogenesis, altering levels of IL-1 β and hippocampal atrophy, among others.

Stress as described earlier, induces secretion of IL-1β from immune cells of brain and periphery. Raised levels of this cytokine can alter negative feedback control of glucocorticoid secretion hence proving that there is a bilateral relation between increased IL-1β and HPA axis dysregulation following exposure to stress.

IL-1 β & Hippocampus: Modulation of IL-1β and HPA axis results in deleterious effects on hippocampus. Being the principal region involved in brain neuroplasticity as well as adult neurogenesis, these effects undoubtedly get translated into a deficit in the above mentioned parameters of hippocampal function leading to behavioral consequences as well as learning and memory deficit. Various theories about cellular mechanisms exist as to how IL-1β affects neurogenesis and neural plasticity and LTP.

Elevated concentrations of IL-1β over long periods of time are lethal to newly born neuronal population in dentate gyrus and hippocampus. Various stages of neurogenesis have been found to be sensitive to

unchecked IL-1β and glucocorticoid concentrations (Wu, M. D., Hein, A. M., Moravan, M. J. *et al*, 2012). Bacterial agent lipopolysaccharide as well as exposure to radiation, which acts by promoting IL-1 β and other inflammatory markers result in decreased neurogenesis (Ekdahl, C. T., Claasen, J. H., Bonde, S. *et al*, 2003c;Monje, M. L., Toda, H., and Palmer, T. D., 2003a). Goshen et al. also suggested role of this cytokine in mediating depressive like effect of chronic stress in rodents by showing behavioral modification, increase in hippocampal levels of IL-1 β as well as decreased neurogenesis in rodents exposed to chronic mild stress (Goshen, I., Kreisel, T., Ben-Menachem-Zidon, O. *et al*, 2008). Role of IL-1 β in mediating these effects is critical as shown by Koo et al. in their experiment. Koo et al. tested stress paradigm for its effects on neurogenesis and its behavioral outcomes using IL-1 receptor antagonist as well as a transgenic strain of mice knock out for IL-1β receptor. Their results suggest that IL-1β receptor is essential for antineurogenic and anhedonic effects of stress. This study highlighted the fact that modulation of IL-1 β activity blocks antineurogenic effects of stress. They also demonstrated that stress suppresses cell proliferation via IL-1β via acting on IL-1RI and blocking this pathway, either by using an inhibitor or by developing transgenic mice lacking this receptor blocks antineurogenic effects. This effect is critical to the behavioral outcome of stress as blocking this effect will result in blockade of behavioral modifications brought about by stress. They also concluded that IL-1β suppresses proliferation of hippocampal neuronal progenitor cell by arrest of cell cycle or both cellular death and arrest of cell cycle (Koo, J. W. and Duman, R. S., 2008). In another experiment, overexpression of IL-1RI resulted in lack of any negative effect on neurogenesis or gliosis (Spulber, S., Oprica, M., Bartfai, T. *et al*, 2008). Antineurogenic effects of chronic stress were completely switched off in rodents transplanted intrahippocampally with transgenic neural precursor cells overexpressing IL-1RI by undoing the otherwise elevation observed with such stress in wild type mice (Ben Menachem-Zidon, O., Goshen, I., Kreisel, T. *et al*, 2008). In addition to the direct toxic effects on new neurons, activation of kynurenine pathway leading to decreased neurogenesis and increased neurodegeneration has also been proposed as a possible mechanism of action for detrimental effects of Il-1β on neurogenesis and its behavioral outcomes (Zunszain, P. A., Anacker, C., Cattaneo, A. *et al*, 2012).

2.2.2.2. Other pro-inflammatory cytokines

In addition to IL-1β pro-inflammatory cytokines include IL-6 and TNF alpha. They are acute-phase proteins which means they are found in response to an acute immune challenge (Dofferhoff, A. S., Vellenga, E., Limburg, P. C. *et al*, 1991;Koj, A., Magielska-Zero, D., Bereta, J. *et al*, 1988). Peripherally, IL-6 is secreted by macrophages and monocytes to stimulate differentiation and proliferation of B cells (Mayer, P., Geissler, K., Valent, P. *et al*, 1991;Hodgkin, P. D., Bond, M. W., O'Garra, A. *et al*, 1988). Activated microglia employ IL-6 as a key antineurogenic signal (Monje, M. L., Toda, H.,

and Palmer, T. D., 2003b), which can interact directly with neural progenitor cells via IL-6 receptors (Nakanishi, M., Niidome, T., Matsuda, S. *et al*, 2007). IL-6 levels also correlate with cognitive performance, predicting memory impairment when elevated (Elderkin-Thompson, V., Irwin, M. R., Hellemann, G. *et al*, 2012;Marsland, A. L., Petersen, K. L., Sathanoori, R. *et al*, 2006). Tumor necrosis factor-alpha is secreted by macrophages, mast cells, and natural killer cells, which stimulates release of other mediators of inflammation like prostaglandins and other pro-inflammatory cytokines (Lindemann, R. A., 1991). TNFα has been studied for its potential role in behavioral modifications. It has been found that its administration leads to an increase in immobility time in tail suspension and forced swim test, both of which depict a depressive like state in mice (Kaster, M. P., Gadotti, V. M., Calixto, J. B. *et al*, 2012). The same study also reported an antidepressant like profile exhibited by TNF and it was found that two transgenic mice strain knockout for TNF receptor 1 and receptor 2 ($TNFR1^{-/-}$,$TNFR2^{-/-}$)showed an antidepressant like effect in forced swim test in both the strains while $TNFR2^{-/-}$ mice showed higher sucrose consumption than control mice and $TNFR1^{-/-}$ mice showing less freezing on fear conditioning test, which collectively implies a strong role of TNF in modulation of depressive like behavior (Simen, B. B., Duman, C. H., Simen, A. A. *et al*, 2006). Similarly, TNF-alpha has appreciable antiproliferative activity on neuronal progenitor cells via TNF receptor 1 (TNF-R1) receptors (Liu, Y. P., Lin, H. I., and Tzeng, S. F., 2005;Cacci, E., Claasen, J. H., and Kokaia, Z., 2005;Iosif, R. E., Ekdahl, C. T., Ahlenius, H. *et al*, 2006). Still other members of this group of cytokines are IL-2, IL-8, IL-12 and IFN-gamma but their role has been explored in very few studies and doesn't constitute a base to characterize their participation in the neuroinflammatory process.

2.2.2.3. Anti-inflammatory cytokines

Role of anti-inflammatory cytokines in physiology and pathology of behavior has not been explored in great detail and only a handful of data is available on this subject. IL-4 and IL-10 are two principal anti-inflammatory cytokines. Of available scientific literature on this subject, evidence has been shown that IL-10 protects the subject against most of the inflammatory stimuli, for instance, experimental autoimmune encephalitis (EAE) (Cua, D. J., Groux, H., Hinton, D. R. *et al*, 1999), meningitis (Koedel, U., Bernatowicz, A., Frei, K. *et al*, 1996), cerebral ischemia (Spera, P. A., Ellison, J. A., Feuerstein, G. Z. *et al*, 1998). Administration of IL-10 prior to LPS injection prevents the negative behavioral effects of LPS injection (Bluthe, R. M., Castanon, N., Pousset, F. *et al*, 1999), including the effects upon mobility, rearing activity and social exploration and interaction (Leon, L. R., Kozak, W., Rudolph, K. *et al*, 1999;Nava, F., Calapai, G., Facciola, G. *et al*, 1997;Smith, E. M., Cadet, P., Stefano, G. B. *et al*, 1999). IL-10 inhibits production of TNF alpha from human monocytes activated by LPS (Shin, D. I., Banning, U., Kim, Y. M. *et al*,

1999). A possible explanation of such effects is that IL-10 exerts an inhibitory effect on IL-1, INF-c and TNF production and does not act as an anti-inflammatory molecule; in fact, it has been shown that IL-10 is important on the down-modulation of these pro-inflammatory cytokines (Fiorentino, D. F., Zlotnik, A., Mosmann, T. R. *et al*, 1991;Harvey, D., Smith, R., English, K. *et al*, 2006;Moore, K. W., de Waal, Malefyt R., Coffman, R. L. *et al*, 2001). Decreased IL-10/IFN gamma ratio has been found to correlate with depressive illness and it is reversed in patients treated with antidepressants (Kubera, M., Lin, A. H., Kenis, G. *et al*, 2001). However, more recent data regarding its potential effects on an animal's behavior show that IL-10 administration without exposure to inflammatory challenge induces increased motor activity and abnormal exploratory patterns in the subject (Harvey, D., Smith, R., English, K., Mahon, B., and Commins, S., 2006), which is suggestive of its own influence on behavior. A study of transgenic mice expressing and lacking IL-10 reveals that knockout mice for IL-10 showed depressive like behavior as compared to wild type mice and mice lacking IL-10 but getting IL-10 injections. Another study using IL-$10^{-/-}$ mice reported elevation in the basal concentrations of tumor necrosis factor alpha as well as IL-6, two of the principal pro-inflammatory cytokines (Agnello, D., Villa, P., and Ghezzi, P., 2000). Certain variations of level of the said cytokine were found to affect female mice more than their male counterparts, which coincides with the clinical data showing females to be more susceptible to depressive illness than males (Mesquita, A. R., Correia-Neves, M., Roque, S. *et al*, 2008b). This may in part be due to the fact that in IL-10 knockout mice, cytokine production is increased due to loss of inhibitory effect of estrogen on those inflammatory molecules (Mesquita, A. R., Correia-Neves, M., Roque, S. *et al*, 2008c).

Role of P2X7 receptors in neuroinflammation

P2X7 receptor is a member of purinergic receptor family. It is an ATP activated ligand gated channel which is abundantly expressed by cells of immune lineage as well as neurons inside the brain (Sperlagh, B., Vizi, E. S., Wirkner, K. *et al*, 2006). These are a part of an inflammatory cascade which is initiated by ATP (Kahlenberg, J. M. and Dubyak, G. R., 2004). It has distinct characteristics of transmembrane pore formation on prolonged activation thanks to the long intracellular C terminal domain, which eventually leads to cell death via increased permeability to large molecular weight molecules (Sperlagh, B., Vizi, E. S., Wirkner, K., and Illes, P., 2006). It is also different from rest of its family for having an extracellular loop and two transmembrane domains (Sperlagh, B., Vizi, E. S., Wirkner, K., and Illes, P., 2006). These receptors are expressed by antigen presenting immune cells, predominantly microglia (Sperlagh, B., Vizi, E. S., Wirkner, K., and Illes, P., 2006). Although initially reported to be absent on neurons (Collo, G., Neidhart, S., Kawashima, E. *et al*, 1997a) these receptors are now believed to be expressed by excitable cell population as well and are known to affect neuronal survival and function (Moores, T. S., Hasdemir, B., Vega-Riveroll, L. *et al*, 2005;Deuchars, S. A., Atkinson, L., Brooke, R. E. *et al*, 2001;Brandle, U., Kohler, K., and Wheeler-Schilling, T. H., 1998;Ishii, K., Kaneda, M., Li, H. *et al*, 2003;Puthussery, T. and Fletcher, E. L., 2004;Armstrong, J. N., Brust, T. B., Lewis, R. G. *et al*, 2002).

3.1. Introduction to P2X family of receptors

Virtually all cell types express plasma membrane receptors for extracellular nucleotides named P2 receptors. P2X7 receptors belong to family of P2 receptors of which, 15 members have been cloned and classified into ionotropic P2X (ligand-gated ion channel receptors) or metabotropic P2Y (G-protein coupled receptors) receptor families (Abbracchio, M. P. and Burnstock, G., 1994;Burnstock, G. and Kennedy, C., 1985;Fredholm, B. B., Abbracchio, M. P., Burnstock, G. *et al*, 1994). P2X receptors function as ATP-gated nonselective ion channels permeable to Na+, K+, and Ca2++ and are further classified into seven subtypes, $P2X_1$ – $P2X_7$ based on protein subunits encoded by 7 different P2X receptor genes (*P2X1* through *P2X7*) expressed in mammalian and other vertebrate genomes. Most P2X receptors are expressed in excitable or epithelial/endothelial tissues; their multiple roles in Ca^{2+}-based signaling responses in these tissues is based on their control over conductance for Ca^{2+} influx or indirect activation of voltage-gated Ca^{2+} channels. These receptor subtypes exist naturally as both homo- and hetero-oligomers with P2X6 and P2X7 being exception for not being able to make homo-oligomers and heterotrimeric channels with other sub-units respectively (Kaczmarek-Hajek, K., Lorinczi, E., Hausmann, R. *et al*, 2012). The 7 isoforms share a similar structure comprising 2 transmembrane domains, a large extracellular loop containing 10 similarly spaced cysteines and glycosylation sites, and intracellular amino and carboxyl termini. P2X7 receptors are believed to be trimer in their stoichiometric conformation

(North, R. A., 2002;North, R. A. and Surprenant, A., 2000).

P2X7 along with P2X2 are also distinct in having an extra-long C terminus, which has enabled scientists explore their interaction more than others hence most of P2X7 receptor enabling it to partake in multiple processes (Kaczmarek-Hajek, K., Lorinczi, E., Hausmann, R., and Nicke, A., 2012).

P2X receptors are non-selective channels permeable to small monovalent and divalent cations. Activation of these receptors alters membrane potential which leads to subsequent cellular events like modulation of neurotransmitter release pre-synaptically and fast excitatory signaling post-synaptically (Sperlagh, B., Heinrich, A., and Csolle, C., 2007;Khakh, B. S., 2001). In addition, they can also modulate the intracellular concentration of Ca^{++} both by increasing permeability to Ca^{++} ions as well as by assisting voltage gated Ca^{++} channels (Shigetomi, E. and Kato, F., 2004;Koshimizu, T. A., Van, Goor F., Tomic, M. *et al*, 2000), making them capable of participating in wide range of intracellular events through activation of second messenger system even at low membrane potentials when highly Ca^{++} permeable NMDA receptors remain inactive (Pankratov, Y. V., Lalo, U. V., and Krishtal, O. A., 2002). In response to activation by bindings of ligands like ATP, the receptors open up channels and increase efflux of ions. As discussed already *(IL-1β)*, this pore formation is critical to the well-being of the cell when it involves P2X7 receptor.

3.2. The P2X7 receptor

P2X7 receptors are important member of immune regulation. Their role in cytokine modulation and neurological disorders is a rapidly expanding area of research. ATP acts as a neurotransmitter as well as a marker of cellular injury while its action on P2X7 receptor has serious cytotoxic consequences. In view of their key status in cytokine production and release, P2X7 receptors are undoubtedly an important player in regulation of neuronal cell death in response to pathological insults.

P2X7 receptor has been implicated in a broad range of functions, which include lymphocyte proliferation (Greig, A. V., Linge, C., Cambrey, A. *et al*, 2003), fertilization, giant cell formation, sleep regulation (Krueger, J. M., Taishi, P., De, A. *et al*, 2010), killing of invading mycobacteria (Lees, M. P., Fuller, S. J., McLeod, R. *et al*, 2010), IL-1 post-translational processing (Ferrari, D., Pizzirani, C., Adinolfi, E. *et al*, 2006), neurotransmitter modulation and subsequently modulating synaptic activity as well as neuron-glia signaling (Duan, S., Anderson, C. M., Keung, E. C. *et al*, 2003), microglial activation, neuroinflammation and neurodegeneration apoptosis and cell death (Abbracchio, M. P. and Burnstock, G., 1998).

3.2.1. Localization of P2X7Rs/vascular immune…..

This receptor is abundant in cells of immunological origin or function, in

particular macrophages (Steinberg, T. H., Newman, A. S., Swanson, J. A. *et al*, 1987b), mast cells (Cockcroft, S. and Gomperts, B. D., 1979), and microglia (Visentin, S., Renzi, M., Frank, C. *et al*, 1999). Bacterial antigen lipopolysaccharide is believed to bind close to its carboxy terminal by virtue of which a potential stimulus is translated into a cascade of inflammatory reaction (Denlinger, L. C., Fisette, P. L., Sommer, J. A. *et al*, 2001).

3.2.2. Mechanism of activation/Activating factors

IL-1 β has been proposed to be the chief cytokine in the brain when it comes to behavior altering effects of stress (Koo, J. W. and Duman, R. S., 2008). Production of pro- IL-1β from immune cells and its subsequent conversion into its active component IL-1β is dependent upon functioning p2x7 receptors. A study with macrophages obtained from $P2X7R^{-/-}$ mice displayed normal amounts of pro-IL-1β to be produced in response to an inflammatory stimulus but in response to a secondary stimulus (ATP) it failed to show an appropriate conversion of pro IL-1β to its active IL-1β form as well as a subsequent rise in concentration of IL-6, which signifies the importance of functioning p2x7 receptors for inflammatory cascade (Solle, M., Labasi, J., Perregaux, D. G. *et al*, 2001).

P2X family shares ATP and BzATP as agonists (Hattori, M. and Gouaux, E., 2012;Bhargava, Y., Rettinger, J., and Mourot, A., 2012), out of which P2X7R is distinct from other members because it requires significantly higher concentration of ATP (more than 1 mM) to achieve activation, whereas much lower concentration of ATP (<100 micro M) is required to activate other members of the family. Requirement of significantly higher concentration reflects the fact that ATP acts as a ligand on P2X7 receptor. Apart from that, the transmembrane channels formed by P2X7 receptors in response to ATP binding rapidly transform into pores while all other P2X receptors demonstrate non-selective channel-like properties following ligation, P2X7 receptors associated pore formation thus allows passage of hydrophilic moieties as large as 900 Da, making them significant for life and death of the cell (Steinberg, T. H., Newman, A. S., Swanson, J. A. *et al*, 1987a;North, R. A., 1996). Molecular mechanisms involved in this process are poorly understood although theories regarding participation of carboxy-terminal domain of P2X7R have been proposed by domain swapping and deletion experiments (Surprenant, A., Rassendren, F., Kawashima, E. *et al*, 1996;Rassendren, F., Buell, G. N., Virginio, C. *et al*, 1997); this carboxyl-terminal domain of the P2X7R is significantly longer than the comparable domains in the other P2X receptors (Rassendren, F., Buell, G. N., Virginio, C., Collo, G., North, R. A., and Surprenant, A., 1997). Transmembranous cytolytic pore leads to cell death due to escape of large weight molecules (Di, Virgilio F., 1995;Murgia, M., Pizzo, P., Steinberg, T. H.

et al, 1992). The debate on whether the receptor itself is a part of the said cytolytic pore or it just favors pore formation is still on (Schilling, W. P., Wasylyna, T., Dubyak, G. R. *et al*, 1999).

3.2.3. P2X7R and Microglial activation

P2X7 receptor is documented to participate in all kinds of inflammatory and immune reaction in brain including microglial activation in response to an injurious stimulus, ischemia, necrosis as well as apoptosis.

Two documented findings support the link between microglial activation and P2X7R expression level. Significantly increased microglial activation is an obligatory finding in the setting of neuroinflammatory as well as neurodegenerative diseases such as Alzheimer's disease, Parkinson's disease and multiple sclerosis (Benveniste, E. N., 1997;McGeer, P. L. and McGeer, E. G., 1998;Streit, W. J., 2004;McLarnon, J. G., Ryu, J. K., Walker, D. G. *et al*, 2006a). The same clinical conditions are reported to exhibit intense P2X7R expression in the neuroinflammatory foci (Collo, G., Neidhart, S., Kawashima, E. *et al*, 1997b;Parvathenani, L. K., Tertyshnikova, S., Greco, C. R. *et al*, 2003;McLarnon, J. G., Ryu, J. K., Walker, D. G. *et al*, 2006b). It is yet to be confirmed though which of these factors precedes the other.
Second set of evidence comes from the fact that inhibitors of microglial activation also inhibit P2X7R expression. Indirectly, P2X7 receptor antagonism has been shown to improve recovery and cell survival following spinal cord injury (Wang, X., Arcuino, G., Takano, T., Lin, J., Peng, W. G., Wan, P., Li, P., Xu, Q., Liu, Q. S., Goldman, S. A., and Nedergaard, M., 2004). Similarly, transgenic mice models lacking P2X7 receptors have shown decreased severity of inflammatory reactions by altering the production of various pro-inflammatory cytokines (Solle, M., Labasi, J., Perregaux, D. G., Stam, E., Petrushova, N., Koller, B. H., Griffiths, R. J., and Gabel, C. A., 2001).

3.2.4. P2X7Rs and post-translational processing of IL-1β

So the question remains as how do exactly P2X7 receptors participate in post-release modulation and activation of IL-1β? The mechanism is a poorly understood one and only theoretical explanations exist which is shown in figure 12.

In addition to the modulation of IL-1β brought about by extracellular ATP, Gabel et al reported same effect induced by nigericin (Perregaux, D., Barberia, J., Lanzetti, A. J. *et al*, 1992). The commonality between ATP and nigericin is that both decrease concentration of intracellular K^+ to make their effect (Steinberg, T. H. and Silverstein, S. C., 1987a;Perregaux, D., Barberia, J., Lanzetti, A. J., Geoghegan, K. F., Carty, T. J., and Gabel, C. A., 1992). So it was argued that K^+ depletion is the critical step needed to activate caspase-1, also called IL-1β-converting enzyme or ICE, in order to

cleave pro-IL-1β to its active entity, the low molecular weight IL-1β (Budihardjo, I., Oliver, H., Lutter, M. *et al*, 1999). A schematic concept of this processing is shown in figure 12 (taken from (Arulkumaran, N., Unwin, R. J., and Tam, F. W., 2011). The idea is supported by other groups that report blockade of ATP induced secretion release of IL-1β by preventing K^+ efflux (Ferrari, D., Chiozzi, P., Falzoni, S. *et al*, 1997a;Perregaux, D. and Gabel, C. A., 1994b;Ferrari, D., Villalba, M., Chiozzi, P. *et al*, 1996;Sanz, J. M. and Di, Virgilio F., 2000).

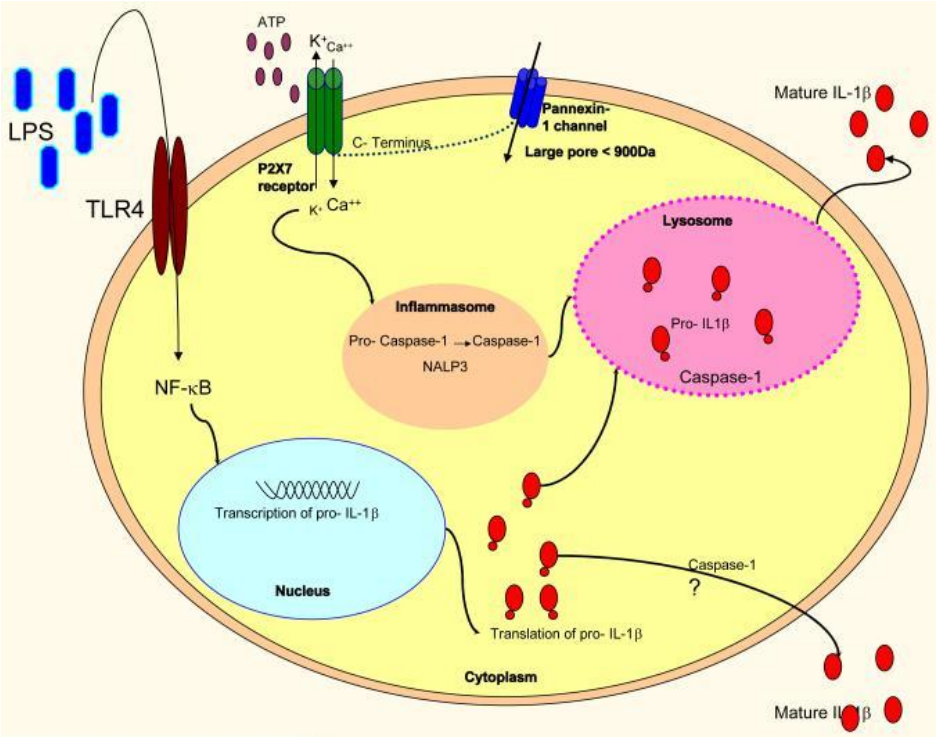

Figure 10: Mechanism of activation of the P2X7 receptor and maturation of Pro-IL-1β to active IL-1β (Arulkumaran, N., Unwin, R. J., and Tam, F. W., 2011)

Importance of role played by P2X7 receptors in this process is supported by the following facts: The agonists and antagonists of P2X7 receptor exert similar effect on consequent release of cytokines (Ferrari, D., Chiozzi, P., Falzoni, S. *et al*, 1997b). Similarly, the antagonist effect of a monoclonal antibody against P2X7 receptor blocks IL-1β release maturation and release (Buell, G., Chessell, I. P., Michel, A. D. *et al*, 1998). Role of P2X7 receptor in providing a fast channel for K+ efflux also signifies its importance in this process (Perregaux, D. and Gabel, C. A., 1994b;Steinberg, T. H. and Silverstein, S. C., 1987b). The mechanism involving K+ depletion on caspase-1 activation is still not understood (Di, Virgilio F., Chiozzi, P., Ferrari, D. *et al*, 2001).

3.3. Role of P2X7 receptors in psychiatric disorders

P2X7 receptor has been implicated in pathophysiology of psychiatric disorders due to SNPs as well as its role in inflammatory cascade in the central nervous system. The gene encoding P2X7 receptor is located at chromosome 12q24 (Buell, G. N., Talabot, F., Gos, A. *et al*, 1998), a region which has been found to be involved in inflammatory and psychiatric disorders through linkage and association studies (Kato, T., 2007;Shen, N. and Tsao, B. P., 2004;Barden, N., Harvey, M., Gagne, B. *et al*, 2006a). Studies in twins as well as first degree relatives have shown some significant relationship. Although environmental factors are also to blame as being common to most of the twins; genetic linkage has been strongly correlated.

3.3.1. P2X7 gene Single nucleotide polymorphisms

A single-nucleotide polymorphism (SNP) is a DNA sequence variation occurring when a single nucleotide — A, T, C or G — in the genome (or other shared sequence) differs between members of a biological species or paired chromosomes in an individual. They are the most common form of genetic variation in the human genome. More than 14 million SNPs have been documented in dbSNP (www.ncbi.nlm.nih.gov/SNP; build 129), the public repository for genetic variation. SNPs are used to detect genetic associations and disease-causing genetic variations. SNPs themselves may lead to alteration in function of a gene. Linkage studies of large families with multiple affected members were used to identify disease causing variations in genes. This approach has led to identification of susceptibility genes for many diseases although a lot of work is required to explore majority of the diseases (Fuller, S. J., Stokes, L., Skarratt, K. K. *et al*, 2009). Several P2X7 gene polymorphisms have been identified to coincide with mood disorders which actually alter the receptor function (Roger, S., Mei, Z. Z., Baldwin, J. M. *et al*, 2010). Although a variety of other genetic variations have also been identified to alter the physiological parameters of mood regulation including polymorphism in promoter region of serotonin transporter gene 5HTTLRP (Lesch, K. P., Bengel, D., Heils, A. *et al*, 1996), in promoter region of IL-1β gene (Borkowska, P., Kucia, K., Rzezniczek, S. *et al*, 2011), the SNPs in P2X7 receptor coding gene generate special interest due to several reasons which are discussed in the following pages (Hejjas, K., Szekely, A., Domotor, E. *et al*, 2009;Lucae, S., Salyakina, D., Barden, N. *et al*, 2006).

The *P2X7* gene is localized on chromosome 12q24.31 (Buell, G. N., Talabot, F., Gos, A., Lorenz, J., Lai, E., Morris, M. A., and Antonarakis, S. E., 1998). It is a highly polymorphic gene. It has 32 non-synonymous amino acid altering identified SNPs. These SNPs may lead either to loss or gain of function mutations. The genomic structure of $P2X_7$ consists of 13 exons, with exon

12 and exon 13 coding for the C-terminal tail of this molecule.

Amino acid asparagine replaces isoleucine in the SNP rs1653624 at residue 568 which results in abnormalities of trafficking and normal cell surface membrane expression of the receptor (Fuller, S. J., Stokes, L., Skarratt, K. K., Gu, B. J., and Wiley, J. S., 2009;Wiley, J. S., Dao-Ung, L. P., Li, C. *et al*, 2003). SNP predisposing populations to bipolar affective disorder or depressive illness has also been identified using population studies of mapping and linkage disequilibrium which is (rs2230912) although the functional effect of this SNP on P2X7R is unknown (Barden, N., Harvey, M., Gagne, B. *et al*, 2006b;Lucae, S., Salyakina, D., Barden, N., Harvey, M., Gagne, B., Labbe, M., Binder, E. B., Uhr, M., Paez-Pereda, M., Sillaber, I., Ising, M., Bruckl, T., Lieb, R., Holsboer, F., and Muller-Myhsok, B., 2006). SNPs have also been identified for tuberculosis (TB) susceptibility, resistance to infection with Chlamydia trachomatis, and increased fracture risk in post-menopausal women (Fuller, S. J., Stokes, L., Skarratt, K. K., Gu, B. J., and Wiley, J. S., 2009).

Of non-synonymous SNPs of P2X7 gene, important effects on cytokine production and post-production have been observed. Glu^{496} to Ala SNP, which is the best studied one so far, causes impairment of ATP induced release of cytokine interleukin-1β (Sluyter, R., Shemon, A. N., and Wiley, J. S., 2004). Another study, however, has reported a 3-5 fold gain of function with the same SNP when tested in combination with the Ala-348 to Thr polymorphism (rs1718119) located in transmembrane domain 2 of the p2x7 receptor, named as haplotype variant 4 ($P2X_7$-4) (Stokes, L., Fuller, S. J., Sluyter, R. *et al*, 2010). The same study also reported another variant $P2X_72$ along with Ala-348 to Thr polymorphism also exhibited an increase in function. The authors suggested that inheritance of these two highly active haplotypes of the gene can be cause of an individual's predisposition inflammatory and/or psychiatric disorders.

3.3.2. Behavior consequences of P2X7R function modulation

As review of the literature suggests, P2X7R makes a potential point of intervention for stress related behavioral and biochemical modifications. A recent study analyzed behavioral profile of transgenic $P2X7^{-/-}$ mice using two classical animal models of depression/behavioral paradigms, tail suspension test and forced swim tests. The results show that $P2X7^{-/-}$ mice showed significant antidepressant picture when compared to their wild type counterparts. Similarly, the KO mice also exhibited an improved response to antidepressant treatment compared to wild type (Basso, A. M., Bratcher, N. A., Harris, R. R. *et al*, 2009). Another study, however, found a single time stressor insufficient to induce a difference in the resignation profile of mice when a batch of P2X7 knockout mice maintained its baseline mobility on exposure to repeated exposure to forced swim stress as compared to their wild counterparts whose immobility time increased from the baseline although the initial baseline immobility time was noted

to be the same in both the groups (Boucher, A. A., Arnold, J. C., Hunt, G. E. *et al*, 2011). It was concluded from the above mentioned experiment that repeated stressors were required to open up P2X7R dependent mechanisms to come into action. In another study though, a spatial recognition task was assigned to wild type as well as $P2X7^{-/-}$ mice, where KO mice displayed spatial memory impairment in a hippocampal-dependent task, while their performances in an object recognition task were unaltered, along with increased hippocampal IL-1β and c-fos expression in wild type mice (Labrousse, V. F., Costes, L., Aubert, A. *et al*, 2009). Another study with $P2X7^{+/+}$, $P2X7^{-/-}$ as well as P2X7R-antagonist treated $P2X7^{+/+}$ mice led the authors to conclude that P2X7 receptor deletion results in a mood stabilizing phenotype when the animals are exposed to different challenges and stress. They also argued that treatment with a selective P2X7 receptor antagonist results in the same profile as receptor deletion which potentially highlights the reversal of depressive-like behavior (Csolle, C., Ando, R. D., Kittel, A. *et al*, 2012). These data highlight the importance of potential benefits of modulation of P2X7 receptor function and open a new window to understand the pathology of the disease as well as suggest newer and potentially more effective ways to intervene.

In view of the fact that psychological stress induced microglial activation doesn't necessarily confer a cellular injury, and subsequently amount of extracellular ATP must remain limited, question arises as how to explain the activation of P2X7 receptor and implication of its polymorphisms? The answer lies with the role of the receptor in modulation of neurotransmitters and neuronal plasticity and variations of this function by the SNPs of the receptor that render unexpected changes in the functionality of the receptor. This hypothesis was presented by Maxwell R. Bennett in 2007 which explains how ATP self maintains a higher concentration needed for the receptor activation and how a polymorphism can potentially disrupt the balance of homeostatic conditions (Bennett, M. R., 2007). This is discussed as under.

Apart from injured cells and tissues. ATP also acts as a signaling molecule for astrocytes mediated Ca++ wave propagation, being released from astrocytes both in spinal cord (Salter, M. W. and Hicks, J. L., 1994) as well as hippocampus (Dani, J. W., Chernjavsky, A., and Smith, S. J., 1992). Its release from astrocytes occurs under the influence of glutamate and is potentiated by substance P (Werry, E. L., Liu, G. J., and Bennett, M. R., 2006). Once extracellular, the ATP can establish a control mechanism for its own release which is independent of glutamate (Bennett, M. R., 2007). Once it reaches sufficient concentration to activate P2X7 receptors on nerve terminals, it favors more glutamate release which in turn facilitates even more ATP, thus creating a feedback loop for ATP. A mechanism of activation of ecto-ATPase is in place to check this rise in ATP concentration (Joseph, S. M.,

Buchakjian, M. R., and Dubyak, G. R., 2003). ATP invites microglia to the site both by acting like a chemoattractant as well as inducing the release of monocyte chemoattractant protein-1 (MCP-1) from astrocytes which migrate physically to the site and along with presynaptic and postsynaptic neurons and astrocytes, give rise to quadpartite synapse (Bennett, M. R., 2007). As presented in figure 13, synaptic microglial P2X7 receptors favor the release of pro-inflammatory cytokine TNF-α under the influence of raised concentration of ATP, which alters synaptic function by increasing number of a-amino-3-hydroxyl-5-methyl-4-isoxazole-propionate (AMPA) receptors for glutamate in post synaptic membrane processes. Inevitably prolonged by the continued activation of the triggering mechanisms, this increase in postsynaptic AMPA receptors leads to permanent changes in neuronal network functions. P2X7 receptor activation also leads to increased IL-1 β which reduces the number of postsynaptic AMPA receptors and a relative concentration of these agents determines the eventual fate of the neural function (Bennett, M. R., 2007). While a balance between the opposing forces is kept under physiological conditions, it is evident that a single nucleotide polymorphism of P2X7 receptor that alters normal functioning of the receptor will inevitably lead to alteration in the balance and consequently a deranged synaptic as well as neural network function, which ultimately implicates the SNPs of P2X7 receptor in neuropsychiatric disorders (Bennett, M. R., 2007).

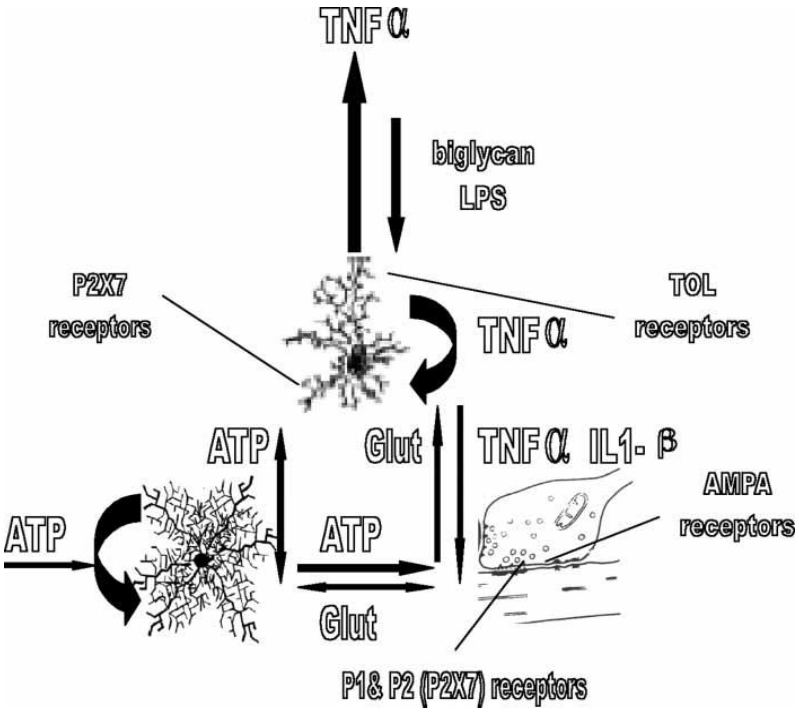

Figure 11: Mechanism of microglial P2X7 receptor activation in presence of ATP and its interaction with pro-inflammatory cytokines. It is obvious that in a finely balanced environment, a genetic variation in form of an SNP which alters the balance. (Bennett, M.R., 2007).

Defining the role of P2X7 receptors in depression: Objectives and hypothesis

What we know about the pathophysiology of depression is that the mood alterations co-exist with biochemical, hormonal as well as inflammatory alterations but the question remains as which of these components is determinant of the other. In a pursuit of finding a cure, disease is explored in detail including its components that overlap with other pathological processes. It enables us to see all the dimensions of a pathological process. Consequences are attributed to causes and points of interventions are identified and exploited for treatment. In experimental paradigms though, many of these processes exist bilaterally which hinders separating cause from consequence and makes it difficult to define a point of intervention. Moreover, study of chemical reactions and equations hardly answers the pertinent question of why certain amongst us can handle physically and psychologically challenging conditions better than others.

The other strategy aims to explore where the story begins, to find the first step in the whole cascade which differentiates a potentially vulnerable person from a resilient person. While research to find a meeting point between various factors affecting mood and behavior has yielded extremely useful data regarding understanding the mechanism of induction of symptoms, it is the finding of genetic predispositions in the form of single nucleotide polymorphisms which has shown a real window of opportunity to find a cure thanks to research according to the later strategy. The findings of the variations in gene expressions that make an individual vulnerable to say for example, environmental and metabolic insults and resulting episodes of depressive illness can explain exactly the opposite in another person who is free of those predisposing genetic elements and manages the same stressors without facing much of a breakdown of mood and personality. Based on these variations of gene expressions for certain proteins which are potentially responsible for predisposing us to disease processes, we can define a person with a particular set of genetic make-up who may be at risk of developing depression if encountered with a psychological or physical stress. Identification of these genetic variations has generated immense interest among biologists. Exploiting them would make room for newer, hopefully safer and more effective agents. Preclinical studies have shown some promises for exploring them as the key holders of relatively less-understood pathologies as well as for their potential to become therapeutic targets.

Neuroinflammation, for instance, is being explored for what it is preceded by and what it is followed by. Its relationship with other determinants of behavior is under scrutiny too. The demarcation line between what separates a protective part of inflammation from a detrimental also remains to be found. Since most of the times such processes are multifactorial, the extent of contribution to injury needs to be dug out as well. Role of inflammation in limiting the injurious stimulus, in contributing

to the ongoing injurious process, in healing and repair, and in manipulation of subject's response to therapeutic interventions need to be defined. Focusing on the behavioral outcome, we need to explore all these dimensions of inflammatory process especially its contribution to the drug response.

Cytokines, as previously stated, are important players in inflammatory process in brain as well as in the periphery. Consequently they have a say in the subject's behavior. To be more precise, it is actually the balance between pro-inflammatory and anti-inflammatory ones that determines the eventual consequence of the ongoing process. It is, thus, quintessential to explore dynamics of these cytokines and the processes that translate them into clinical manifestations in order to find a breakthrough. Determined by the P2X7R and microglial function and followed by behavioral penalties, IL-1β definitely deserves all the attention and interest that it is generating.

Based on this strategy, employing the literature reviewed in previous section regarding inflammatory mechanisms participating in the pathophysiology of depressive illness and their genetic modulation through gene expression, the research work of this thesis explores the link between the depression and neuroinflammation by characterizing the role of P2X7 receptors in the induction of microglial activation/neuroinflammation and recovery process using an animal model of depression called UCMS.

The choice of the animal model was based on the ability of the said model in fulfilling the flagship criteria of validity of animal models of depression proposed by Willner as well as nine criteria proposed by Belzung and Lemoine. Apart from the unsolved parts of the puzzle like the mechanism of induction of behavioral and biochemical changes induction in vulnerable organisms, all major criteria are fulfilled by this model of depression and it stands at the top of the list in all respects. Despite being a rigorous and difficult to build model, it has gained wide respect and acceptance in biological research. It includes exposure of a specific strain of mice, to variety of low intensity social and environmental stressors over a period of several weeks. Exact detail of the protocol is given in the section on material and methods for individual experiments carried out during the course of this thesis.

Taking into account this model of depression, our first experiment was designed to explore the possibility of finding an evidence of microglial activation as a function of exposure of the specific strain of mice to randomized mild social and environmental stressors over a span of nine weeks. The results of this experiment led us to believe that microglial activation in different parts of mice brain was a part and parcel of the depressive like behavior in mice. It didn't, however, elicit a generalized inflammatory response as an analysis of pro-inflammatory cytokines in

peripheral blood was inconclusive. These results were published in Behavioral Brain Research and are presented in the section on results under the Article 1 section.

Encouraged by these results, we conceived our second experiment which was aimed to explore the role of an antagonist of P2X7 receptor in parallel with a conventional antidepressant in reversing the depressive-like phenotype induced by UCMS in the same strain of mice. The results of this experiment show that inhibition of P2X7 receptors leads to reversal of depressive like effects both at behavioral as well as biochemical level, showing a correction of HPA axis alterations brought about by stress exposure. In contrast to antidepressant Fluoxetine used, the P2X7 receptor antagonist did not, however, result in the augmentation of neurogenesis in mice brain as a mechanism of reversal of stress induced changes.

Results

Validation of UCMS model to study neuroinflammation

Inflammatory character has been the latest turn in the story of depressive illness. Increasing amount of data from epidemiological as well biological research suggest that inflammation plays a major role in the induction of neurobehavioral modifications in a susceptible subject. Inflammatory factors have become more visible due to the added finding of induction of depressive symptoms with immunotherapy as well as evidence of enhanced inflammatory markers in individuals suffering from neurodegenerative disorders. The notion that presence of longstanding inflammation can herald degeneration has attracted a lot of attention and more emphasis has been on the earliest presentation of the same i.e. neuroinflammation and depressive illness.

Biological research has contributed immensely to the understanding of its underlying mechanisms. Various types of animal models have been used to explore these processes. They have been successful in identifying pharmacological points of interventions. Ethical concerns involved in experimentation with human tissues as well as in vivo studies have forced biologists to envisage animal models of affective disorders.

Unpredictable chronic mild stress (UCMS) is one such model. Willner and colleagues originally described it in rats. Its adoption in mice has been proved after repeated experiments carried out in order to identify the vulnerable strain as well as other validity criteria. Its protocol involves chronic exposure of a vulnerable strain of mice to social and environmental stressors. These include social and environmental stressors of moderate severity. They are randomized in order to reduce their predictability and have been reproduced over and over again in our setting as well as elsewhere. These effects are reversible when the animals are chronically treated with antidepressants. Many reviewers have found this model as one of the most respected and validated animal model of depression.

When we aimed to study the relationship between depression and neuroinflammation using USCMS as a model, we needed to verify whether this model is valid to do the same. In other words we needed to know whether a UCMS induced depressive like behavior is accompanied by neuroinflammation or not, and if yes, hoe much is the extent of this component. To get an answer to these questions we subjected a group of mice to UCMS for nine weeks and evaluated its effects by quantifying the pharmacologically validated coat state index. The mice were injected with the bacterial inflammatory antigen lipopolysaccharide (LPS), which is a known activator of microglia before being sacrificed. Paraformaldehyde was used to perfuse the mice in order to fix the tissues. Brains were harvested and immunohistochemistry was done for microglial activation. We also managed to analyze the pro-inflammatory cytokines in peripheral blood.

Our results indicate that exposure to UCMS does induce activation of microglia in stress responsive regions of mice brain but didn't elicit a generalized peripheral inflammatory syndrome. The activation of microglia can thus be implicated in the depressive like behavior of mice exhibited by the UCMS exposed mice. It also implies that despite its limitations of the experiment, UCMS can be a model to study depression-induced neuroinflammation. These results have been published in *Behavioral Brain Research*.

Behavioural Brain Research 231 (2012) 130–137

Contents lists available at SciVerse ScienceDirect

Behavioural Brain Research

journal homepage: www.elsevier.com/locate/bbr

Research report

Is unpredictable chronic mild stress (UCMS) a reliable model to study depression-induced neuroinflammation?

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ARTICLE INFO

Article history:
Received 20 January 2012
Received in revised form 8 March 2012
Accepted 12 March 2012
Available online 20 March 2012

Keywords:
Unpredictable chronic mild stress (UCMS)
Animal model of depression
Microglial activation
Neuroinflammation
Neurodegeneration
Depression

ABSTRACT

Unipolar depression is one of the leading causes of disability. The pathophysiology of depression is poorly understood. Evidence suggests that inflammation is associated with depression. For instance, pro-inflammatory cytokines are found to be elevated in the peripheral blood of depressed subjects. Cytokine immunotherapy itself is known to induce depressive symptoms. While the epidemiological and biochemical relationship between inflammation and depression is strong, little is known about the possible existence of neuroinflammation in depression. The use of animal models of depression such as the Unpredictable Chronic Mild Stress (UCMS) has already contributed to the elucidation of the pathophysiological mechanisms of depression such as decreased neurogenesis and HPA axis alterations. We used this model to explore the association of depressive-like behavior in mice with changes in peripheral pro-inflammatory cytokines IL-1β, TNFα and IL-6 level as well as the neuroinflammation by quantifying CD11b expression in brain areas known to be involved in the pathophysiology of depression. These areas include the cerebral cortex, the nucleus accumbens, the bed nucleus of the stria terminalis, the caudate putamen, the amygdala and the hippocampus. The results indicate that microglial activation is significantly increased in the infralimbic, cingulate and medial orbital cortices, nucleus accumbens, caudate putamen, amygdala and hippocampus of the mouse brain as a function of UCMS, while levels of pro-inflammatory cytokines did not differ among the groups. This finding suggests that neuroinflammation occurs in depression and may be implicated in the subject's behavioral response. They also suggest that UCMS could be a potentially reliable model to study depression-induced neuroinflammation.

1. Introduction

Unipolar depression is the leading cause of disease burden in high-income countries [1]. Initial treatment with conventional antidepressants fails to improve almost one-third of the patients [2] due in part to an incomplete understanding of pathophysiology of the disease. Clinical and preclinical data suggest that depression is associated with the activation of the immune system, which manifests as inflammation. This finding has complicated the pathophysiological picture of the disease [3]. In particular, episodes of depression have been characterized by an increase in the levels of various pro-inflammatory cytokines such as tumor necrosis factor (TNFα) and interleukin-6 which have been concluded to be raised in depressed subjects in a meta-analysis for instance [4]. When challenged with a social stress, mice sustain an increase in cytokines and corticosterone in peripheral blood [5]. Depression-like behavior can also be induced by the peripheral administration of cytokines and alleviated by their antagonists [6] as well as by antidepressant treatment which promote anti-inflammatory cytokine IL-10 [7], for a review, see [8]. Pretreatment with a selective serotonin reuptake inhibitor (SSRI) can also reduce the incidence of depression in patients undergoing interferon immunotherapy [9].

Microglia are brain equivalent of peripheral immune cells i.e., lymphocytes. They are found throughout the brain and constitute the prime group of cells which are activated in response to immune challenge [9,10]. Microglia's activation alters the subject's response to stress. It is beneficial in the beginning, as for long term potentiation and neurogenesis in hippocampus, mediated through

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doi:10.1016/j.bbr.2012.03.020

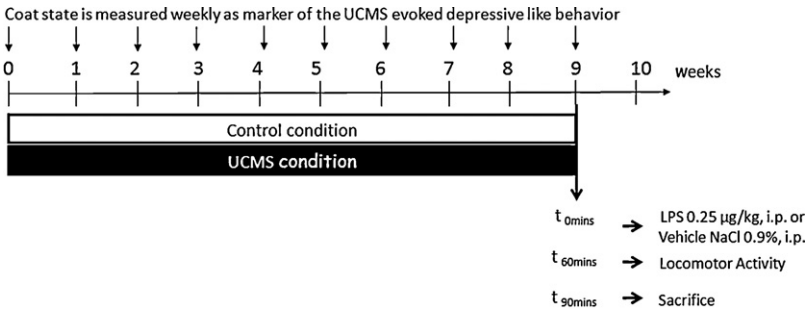

Fig. 1. The experimental protocol showing the weeks during which mice were exposed to UCMS. After 9 weeks of the UCMS or control condition, mice were injected with either LPS (0.25 μg/kg) or NaCl (0.9%) and tested for locomotor activity before being sacrificed. The brains were then collected for immunohistochemistry.

neurotransmitters and inflammatory cytokines like glucocorticoids and IL-1 [11–13]. If, however, it continues for long, it can progress towards neuronal injury and degeneration [11,14]. Any type of stress such as traumatic brain injury [15], cerebrovascular accidents [16], neurodegenerative diseases [17] and infections [18] can lead to microglial activation. Moreover, microglial activation and the resulting neuroinflammation may be implicated in the pathophysiology of neurodegenerative disorders and depressive illness. Increasing epidemiological data suggest a relationship among inflammation, depression and neurodegeneration. Epidemiological studies have shown that depressed subjects are more likely to develop degenerative diseases such as Alzheimer's disease (AD) [19] or Parkinson disease (PD) in older ages [20]. Further preclinical studies suggest that neuroinflammation could be one of the mechanisms implicated in this association. Norepinephrine (levels of which are thought to be decreased in depression) has been shown to suppress Aβ-induced cytokine and chemokine production and to increase microglial migration and the phagocytosis of Aβ [21], while antidepressants such as imipramine [22] have been shown to limit amyloid brain deposition in the mouse. This latter effect is mediated by a decrease in TNF-α expression. Brain imaging studies in humans have shown that depression and/or depressive-like states are associated with morphological (e.g., decreased volume of the hippocampus and increased amygdala volume) as well as functional/molecular brain alterations (such as decreased activation of the temporal cortex and insula; increased activity in the cerebellum, ventromedial prefrontal and anterior cingulate cortices; increased activation of the amygdala; decreased hippocampal neurogenesis; and altered BDNF levels in the nucleus accumbens) [23]. It can therefore be hypothesized that some of these brain changes could be related to neuroinflammatory process and particularly microglial activation. However, at this time, very few studies have attempted to examine the effect of stress-induced microglial activation in the various brain areas known to be implicated in the pathophysiology of depression. Consequently, the objective of this study was to assess the ability of the unpredictable chronic mild stress (UCMS) model, a validated rodent model of depression, to elucidate the role of neuroinflammation in the pathophysiology of depression and any related increase in the risk of neurodegenerative disorders. We therefore sought to measure microglial activation in mice exposed to the UCMS procedure in a subset of brain regions, namely, the cortex (infralimbic, prelimbic, medial orbital, cingulate), nucleus accumbens (core, shell), caudate putamen, amygdala, bed nucleus of the stria terminalis and hippocampus (Cornu Ammonis 1 & 3, dentate gyrus, polymorphous layer and molecular layer of the dentate gyrus). We also compared these results to the effects of bacterial lipopolysaccharide, a well-known activator of microglia. Furthermore, to compare the stress-induced neuroinflammation with the peripheral immune alterations, we measured serum levels of pro-inflammatory cytokines.

2. Material and method

2.1. Animals

Two groups of 7-week-old male BALB/cByJ@Rj mice (Centre d'élevage JANVIER Le Genest-St-Isle France) were subjected to unpredictable chronic mild stress (UCMS) ($n = 14$) or kept in standard housing conditions as controls ($n = 14$) for 9 weeks. Control mice were housed in groups of five, while UCMS mice were housed individually. The light and dark cycle was reversed (i.e., lights on from 8 p.m. to 8 a.m.). Room temperature was maintained at 22 °C ± 2 °C. Food and water were provided ad libitum. All procedures were carried out in the dark phase of the cycle and in accordance with the veterinary service (agreement number C37-261-2), the Ethics Committee for Animal Experimentation (Val de Loire n°2011-06-10), the European Community Council directive 86/609/EEC and the Ministry of Agriculture of France. A general scheme of the experimental protocol is presented in Fig. 1.

2.2. Unpredictable chronic mild stress (UCMS)

It is a variation of the chronic stress procedure described in rats by Willner et al. as a naturalistic rodent model of depression [24]. This protocol consisted of the chronic exposure of mice to various randomly scheduled, low-intensity social and environmental stressors (e.g., social stress, wet bedding, frequent sawdust changes, predator sounds, restraint stress, alterations of the light and dark cycle, tilting of the cages at 45° and the addition of rat droppings to mouse cages). The application of the different stressors was randomized each week to maximize the degree of unpredictability. For ethical reasons, the stress procedure did not involve food or water deprivation. Coat state was measured weekly as one of the markers of UCMS-induced depressive-like behavior [25,26]. The total score given for coat state was the sum of the scores obtained from seven body parts. Two independent, blinded observers performed evaluation of the coat state. After testing inter-observer reliability, the means of the results reported by both observers were statistically analyzed. This index has been pharmacologically validated in previous studies using BALB/c mice. Mice were sacrificed after 9 weeks of exposure to UCMS. The current protocol did not include any antidepressant treatment groups as it was intended to investigate neuroinflammation only.

2.3. Treatment with bacterial antigen lipopolysaccharide (LPS)

Ninety minutes before sacrifice, mice were intraperitoneally injected with the bacterial antigen lipopolysaccharide (LPS) (Sigma–Aldrich MO 63103 USA) at 0.25 μg/kg body weight prepared in 0.9% normal saline at a volume of 10 ml/kg body weight (UCMS $n = 7$ and control $n = 6$). Fifteen mice were injected with normal saline at 10 ml/kg body weight (UCMS $n = 7$ and control $n = 8$). The behavioral effects of LPS were measured by recording locomotor activity one hour after the injection. Locomotor activity was measured for 30 min using an actimeter, which assessed the activity of mice in their home cages. The cage was placed in the center of the device, which consisted of a plane crossed by photo-beam detectors. Movement of the animal was automatically detected and then scored. Higher scores reflected more mouse movement. Transparent cages with 1/3rd of the normal sawdust level were used for this purpose so as to minimize interference with activity detection.

2.3.1. Sacrifice

After the locomotor activity scores were recorded, mice were anesthetized with pentobarbital (injected intraperitoneally at a dose of 40 mg/kg body weight in a volume of 10 ml/kg body weight). Peripheral blood was collected and subjected to centrifugation for 15 min at 5000 rpm. Serum was collected and frozen at −80 °C until the levels of pro-inflammatory cytokines could be measured (Microdosages par la technologie xMAP luminex Plate-forme phénotypage du petit animal et microdosages. Hôpital Saint-Antoine–Bâtiment Kourirsky Paris France). The transcardiac perfusion of mice with 180 ml of 4% paraformaldehyde (PFA) followed. Brains were dissected out, post-fixed in PFA for 2 h and then cryoprotected in 20%

Table 1
The number and bregma level of each of the structures analyzed with respect to the density of microglia. IL *infralimbic cortex*, MOC *medial orbital cortex*, PL *prelimbic cortex*, CGC *cingulate cortex*, CPU *caudate putamen*, NAcC *Nucleus Accumbens Core*, NAcS *Nucleus Accumbens Shell*, BNST *bed nucleus of stria terminalis*, Amyg *amygdala*, CA 1 & 3 *Cornu Ammonis 1 & 3*, DG *dentate gyrus*, MOL *molecular layer of dentate gyrus*, PoDG *polymorphous layer of the dentate gyrus*.

	Structure	Bregma level	Number of sections	Number of pictures per section
Cortex	MOC	2.34 mm to 1.98 mm	2	3–5
	IL	1.98 mm to1.34 mm	2	2
	PL	2.34 mm to 1.54 mm	2	2
	CgC	2.34 mm to −0.10 mm	3	2
Subcortical nuclei	NAcC	1.98 mm to 0.74 mm	3	2–3
	NAcS	1.98 mm to 0.74 mm	2	2
	BNST	0.50 mm to 0.02 mm	2	1
	Amyg	−0.70 mm to −2.06 mm	5	4
	CPu	1.70 mm to −2.18 mm	5	4–6
Hippocampus	CA1	−0.94 mm to −3.52 mm	3	1
	CA3	0.94 mm to −3.52 mm	3	1
	Mol	0.94 mm to −3.52 mm	2	1
	PoDG	0.94 mm to −3.52 mm	1	1
	DG	0.94 mm to −3.52 mm	3	1

sucrose solution at 4 °C. Brains were cut in 40-μm-thick slices on the cryostat (Leica CM 3050S). Every fourth section was collected for immunohistochemistry.

2.3.2. Analysis of inflammatory markers

The serum levels of IL-6, IL-1 and TNFα were studied in duplicate in the same assay by using the multiplex immunoassay kit (Millipore Corporate, Billerica, MA). The samples were diluted to 1:100 and quantified by mean of Antibody-Immobilized beads and Luminex MAP technology (xMAP Luminex IFR65, Paris).

2.3.3. Immunohistochemistry

Free-floating sections were washed in 0.1 M PB, 50% ethanol and 3% H_2O_2. Sections were incubated at room temperature in rat anti-mouse CD11b antibody (AbDSerotec, Oxford, UK; diluted 1:500) for 48 h, followed by three washes in PB and a 2-h incubation in secondary donkey anti-rat IgG biotinylated antibody (Jackson Immuno Research, West Grove, Pennsylvania; diluted 1:500). Sections were then incubated in avidin–biotin–peroxidase complex (Vectastain ABC kit; Vector Laboratories, Burlingame, CA, USA; diluted 1:100) for 1 h and reacted with freshly prepared diaminobenzidine-HCl (DAB; Sigma–Aldrich, St. Louis, Missouri, USA) in the presence of cobalt and H_2O_2. The sections were then rinsed in PB, mounted on gelatinized glass slides, dehydrated, cleared in Claral (Réactifs RAL) and coverslipped with Eukitt®.

2.3.4. Image analysis

Four to six images were taken of each brain structure at different bregma levels (see Table 1 for the exact level and number of photographs for each structure) using the video camera module XC77CE (Sony) with an AF Micro Nikkor 60-mm lens (Nikon). The pictures were analyzed using Histolab software to detect CD11b-expressing cells and calculate the area density of microglia (see Fig. 2). Differences in the area covered by microglia were considered an index of differences in the level of microglial activity. The brain regions assessed in this study included four cortical areas (infralimbic, prelimbic, medial orbital and cingulated cortex), sub-cortical nuclei (the core and shell parts of the nucleus accumbens, the caudate putamen, and the bed nucleus of the stria terminalis and amygdala) and five areas of the hippocampus (CA1, CA3, dentate gyrus and its molecular and polymorphous layers). Fig. 3 shows representative images of CD11b staining in CA1 region of mice brain hippocampus from different groups of mice studied.

2.3.5. Statistical analysis

Non-parametric tests were performed to analyze behavioral, histological and cytokine-concentration data due to the limited number of animals per group. The Kruskal–Wallis test was performed followed by a Mann–Whitney U test including corrections for multiple comparisons when required. Differences were considered significant when the p value was less than 0.05. All data are expressed as the mean ± SEM.

3. Results

3.1. UCMS induces a depression-like state in mice

The results related to coat state are illustrated in Fig. 4. The coat state score shows that UCMS induced a depressive-like state in mice, as demonstrated by significantly higher coat state scores in the stressed group compared to the control group, starting from the second week of stress exposure until week 9 (Group Kruskal–Wallis test 2nd week: H (1, N = 29) =12.20210 p = 0005, 9th week: Group Kruskal–Wallis test: H (1, N = 29) =12.72223 p = 0004).

3.2. LPS decreases locomotor activity in mice

The intraperitoneal injection of LPS induces a profound decrease in the locomotor activity of mice as shown in Fig. 5 (Kruskal–Wallis test: Kruskal–Wallis test: H (3, N = 29) = 17.01602, p = 0007). Both the UCMS and control groups showed a significant decrease in activity after LPS treatment (p = 0.017 and 0.000, respectively). The

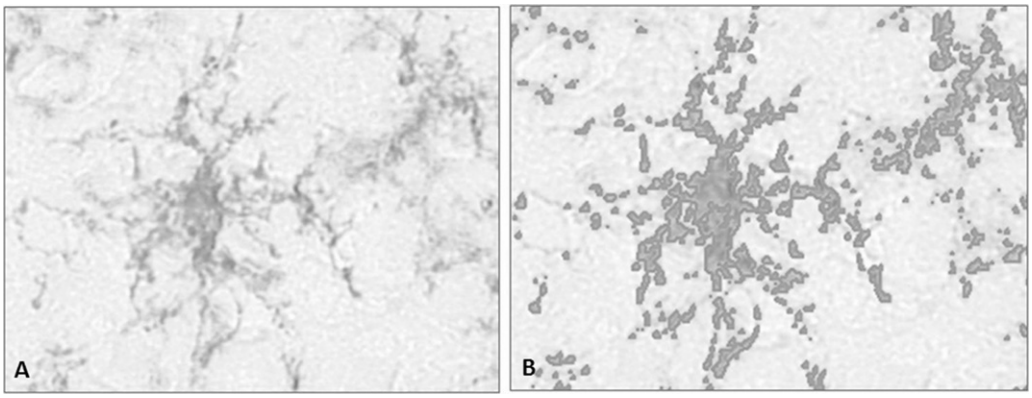

Fig. 2. A magnified single microglial cell (×40) is shown to depict the steps of image analysis used in the calculation of surface area: (A) as photographed by the microscope camera; (B) when subjected to analysis by Histolab software, showing the cell body and branches as detected by the software.

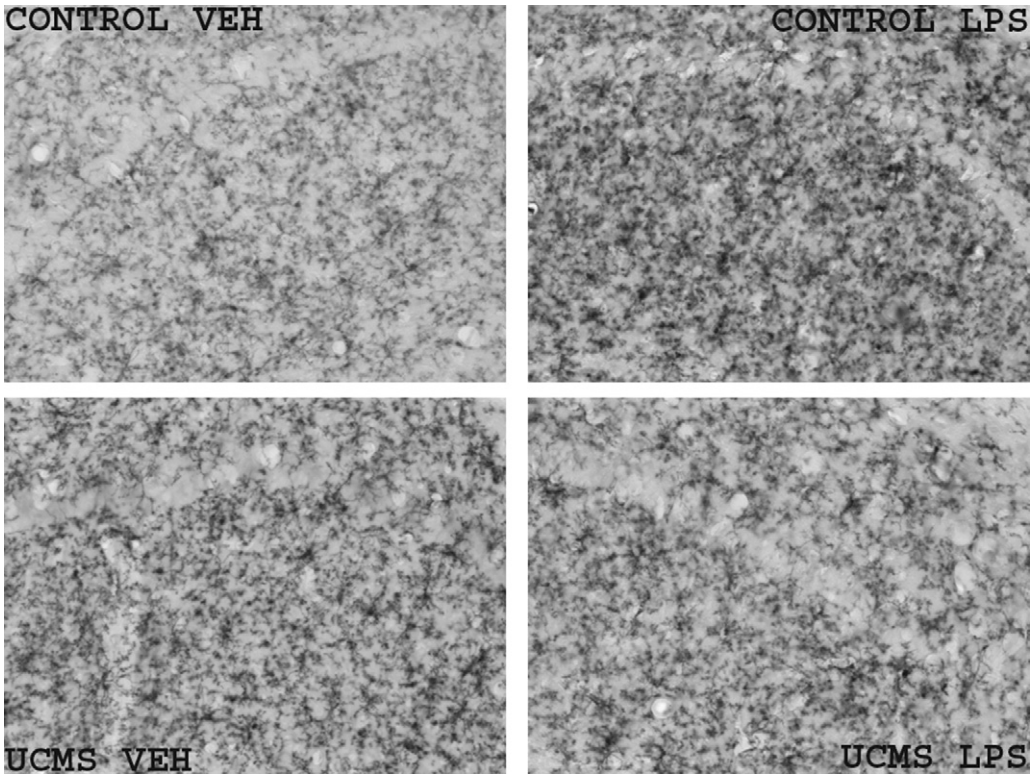

Fig. 3. Representative CD11b staining in CA1 region of mice brain hippocampus exposed or not to unpredictable chronic mild stress (UCMS) and intraperitoneal lipopolysaccharide (LPS) treatment.

UCMS group that was not treated with LPS showed higher activity scores compared to the control group not treated with LPS ($p < 0.04$). Control mice treated with LPS exhibited a significantly lower score of locomotor activity when compared to UCMS mice treated with LPS ($p = 0.02$).

3.3. Analysis of peripheral pro-inflammatory cytokine IL-1β, TNFα and IL-6

The levels of pro-inflammatory cytokines IL-1β, TNFα and IL-6 in blood collected before cardiac perfusion at the end of the experimental protocol were measured. The analyses showed that neither the UCMS model nor the LPS injection modified plasma levels of pro-inflammatory cytokines. These results can be seen in Fig. 6. The Kruskal–Wallis test values were as follows: H (3, $N = 28$) = 3.491829, $p = 3218$ for IL-1β; H (3, $N = 28$) =4.535042, $p = 2092$ for TNFα; and H (3, $N = 28$) = 4.535042, $p = 2092$ for IL-6.

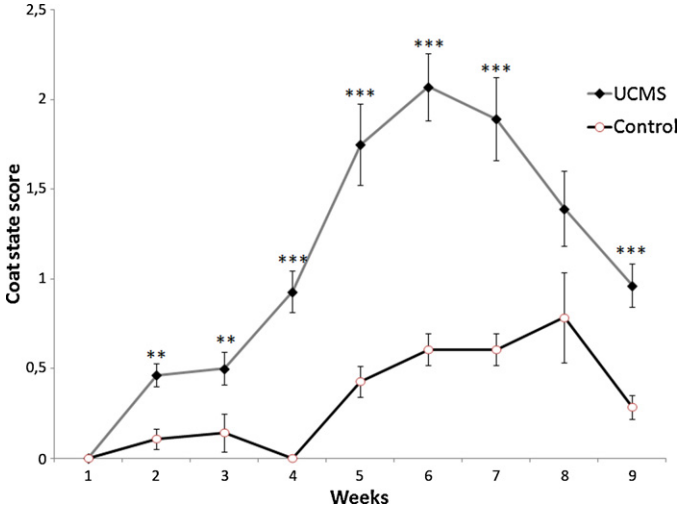

Fig. 4. Comparison of coat state during the 9 weeks of the experiment between the UCMS ($n = 14$) and control groups ($n = 14$). The results show significantly elevated scores in mice subjected to UCMS (week 2-week 9). ** $p < 0.01$, *** $p < 0.001$ Control vs. UCMS.

3.4. UCMS-induced neuroinflammation in stress-responsive areas of the mouse brain cannot be further increased by the use of bacterial lipopolysaccharide

The results of LPS- and UCMS-induced microglial activation can be found in Figs. 7–9. We observed significantly higher microglial activation in different regions of the brain after the

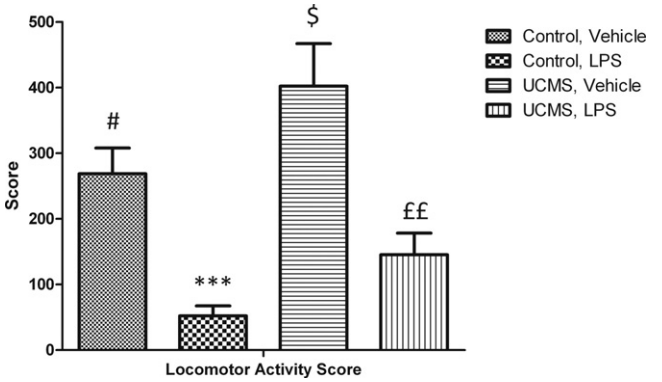

Fig. 5. Comparison of 30-min locomotor activity scores one hour after the intraperitoneal injection of bacterial antigen lipopolysaccharide in the UCMS and control groups after 9 weeks of experimentation. *** $p < 0.001$, Control, Vehicle vs. Control, LPS, # $p < 0.05$ Control, Vehicle vs. UCMS, Vehicle, $$ $p < 0.01$ UCMS, Vehicle vs. UCMS, LPS, ff $p < 0.01$ Control, LPS vs. UCMS, LPS.

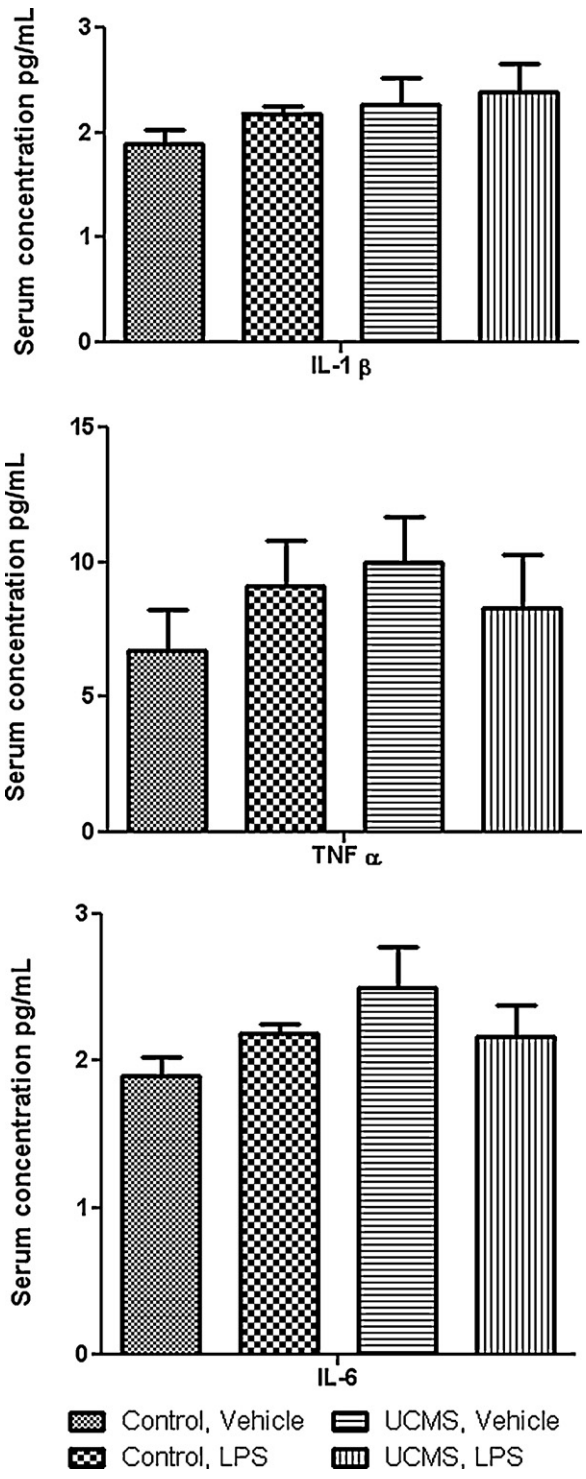

Fig. 6. Serum concentrations of IL-1β, TNFα and IL-6 in the peripheral blood of mice, as measured before cardiac perfusion. Values are the means ± SEM.

use of both UCMS and LPS. The non-parametric Kruskal–Wallis test did not reveal significant differences in the *prelimbic cortex* (*PL*) [H (3, *N* = 26) = 6.812943 *p* = 0781], *cornuammonus 3* (*CA3*)[H (3, *N* = 28) = 7.730208 *p* = 0519], *bed nucleus of the striaterminalis* (*BNST*) [H (3, *N* = 28) = 5.543015 *p* = 1361], *molecular layer of the dentate gyrus* (*Mol*) [H (3, *N* = 28) = 5.260380 *p* = 1537], *polymorphous layer of the dentate gyrus* (*PoDG*) [H (3, *N* = 28) = 5.301284 *p* = 1510], or the *dentate gyrus* (*DG*) [H (3, *N* = 28) = 4.646024 *p* = 1996]. Significant differences were found in the *infralim-*

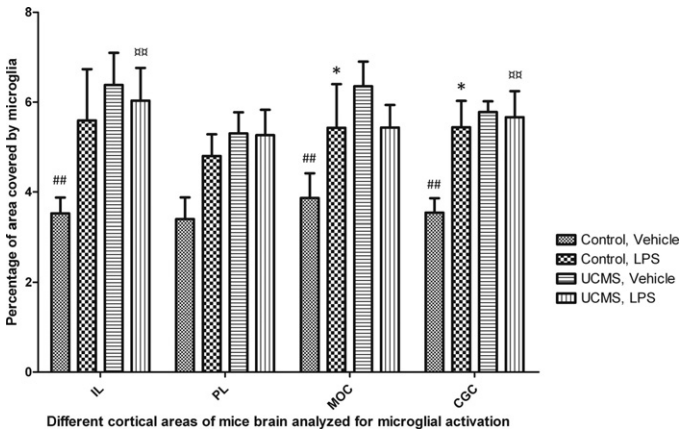

Fig. 7. The area density of microglia in different cortical areas for the UCMS and control groups treated with LPS or vehicle. IL *infralimbic cortex*, MOC *medial orbital cortex*, PL *prelimbic cortex*, CGC *cingulate cortex.* * $p < 0.05$, Control, Vehicle vs Control, LPS # $p < 0.05$, ## $p < 0.01$, Control, Vehicle vs UCMS, Vehicle ¤¤ $p < 0.01$ Control, Vehicle vs UCMS, LPS.

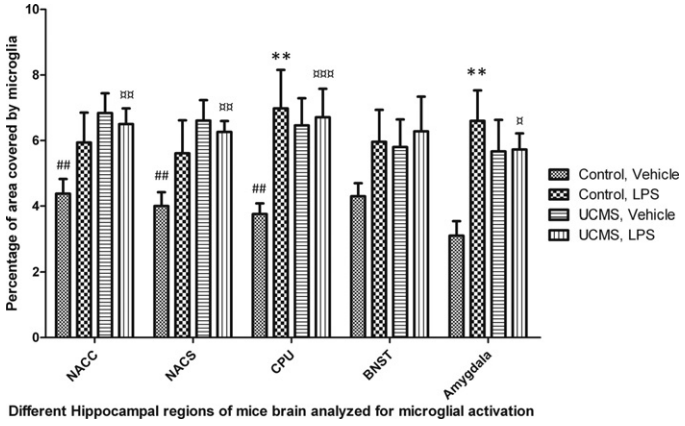

Fig. 8. Different brain nuclei analyzed for microglial activation in the UCMS and control groups treated with LPS or vehicle. CPU *caudate putamen*, NAcC *Nucleus Accumbens Core*, NAcS *Nucleus Accumbens Shell*, BNST *bed nucleus of stria terminalis*, Amyg *amygdala* ** $p < 0.01$ Control, Vehicle vs Control, LPS ## $p < 0.01$Control, Vehicle vs UCMS, Vehicle ¤ $p < 0.05$, ¤¤ $p < 0.01$, ¤¤¤ $p < 0.001$ Control, Vehicle vs UCMS, LPS.

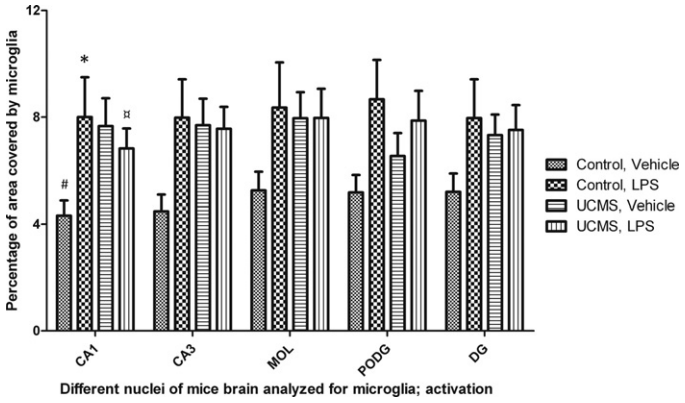

Fig. 9. Microglial activation in different hippocampal regions in the UCMS and control groups treated with LPS or vehicle. CA 1 & 3 *Cornu Ammonis 1 & 3*, DG *dentate gyrus*, MOL *molecular layer of the dentate gyrus*, PoDG *polymorphous layer of the dentate gyrus.*
* $p < 0.05$ Control, Vehicle vs Control, LPS # $p < 0.05$ Control, Vehicle vs UCMS, Vehicle ¤¤ $p < 0.01$Control, Vehicle vs UCMS, LPS.

bic cortex (*IL*) [H (3, $N = 27$) = 10.10408 $p = 0177$], *media orbital cortex* (*MOC*) [H (3, $N = 27$) = 8.898866 $p = 0307$], *cingulate cortex* (*CgC*) [H (3, $N = 28$) = 13.09184 $p = 0044$], *nucleus accumbens core* (NAcC) [H (3, $N = 28$) = 8.601073 $p = 0351$], *nucleus accumbens shell* (*NAcS*) [H (3, $N = 28$) = 10.18121 $p = 0171$], *caudate putamen* (CPu) [H (3, $N = 28$) = 12.94159 $p = 0048$], *amygdala* (Amyg) [H (3, $N = 28$) = 9.485310 $p = 0235$], and *cornusammonus* (CA1) [H (3, $N = 28$) = 8.469388 $p = 0372$]. Further analysis with the Mann–Whitney test revealed significant differences in microglial activation as a function of LPS treatment in IL, MOC, CgC, NAcC, NAcS, CPu and the CA1 region of the hippocampus. UCMS-induced microglial activation was observed in the MOC, CgC, CPu, and Amyg, as well as in the CA1 region of the hippocampus. The UCMS LPS group showed no significant difference in microglial activation when compared to the UCMS Vehicle or Control LPS groups. However, there was a significant difference in microglial activation between the UCMS LPS group and the Control Vehicle group in the IL, CgC, NAcC, NAcS, CPu, Amyg and CA1 region.

4. Discussion

Our findings show that UCMS produced depression-like behavior in mice that was associated with microglial activation in various regions of the mouse brain. This pattern was not accentuated by LPS, although a behavioral sickness syndrome was elicited by this drug, as is evident from a decrease in the locomotor activity observed following its intraperitoneal injection.

The depression-like behavior was quantified by the reduction in coat quality as a function of the exposure to UCMS, as reported in several previous experiments in the same mouse model of depression [27–30]. The improvement in coat state score towards the end of experiment can possibly be due to variation in severity of stressors applied as well as the habituation effect.

Bacterial antigen lipopolysachharide (LPS) is a potent stimulator of microglia [31], promoting survival of activated microglia in low concentration while causing cell death at higher concentration in vitro [32]. Apart from being an acute inflammatory stimulus [33], it is itself known to induce a syndrome of behavioral sickness which is considered as a model of depression [34,35]. The decrease in locomotor activity induced by LPS observed here is in accordance with previously published results [36]. LPS significantly reduced locomotor activity in both the control and UCMS groups, whatever the stress level. Surprisingly however, UCMS induced an increase in this parameter, which has not been observed in previous studies of our group. This can be due to the fact that here activity was recorded during 30 min duration, while in previous studies we used a 4-h duration [26]. This short duration may cause a novelty-related hyper-activity that cannot be observed once the animals have habituated to the situation (during 4 h). In any case, UCMS effects do not parallel LPS effects on this variable, which might mean that these two factors do not elicit the same behavioral profile, even if they are both associated to depressive-like states.

The analysis of pro-inflammatory cytokines in peripheral blood did not indicate an increase related to UCMS or LPS. Absence of cytokine alteration can be attributed to the fact that we used serum instead of plasma for cytokine analysis. However previous studies using UCMS [37], social stress as well as intraperitoneal LPS injection have also reported an absence of cytokine alterations even though the concentration of LPS used was higher (2 μg/kg compared to 0.25 μg/kg that we used) [38]. Furthermore, the peripheral inflammatory effects of this compound may not be discernible 90 min after injection. The absence of increases in serum levels of IL-1β, IL-6 and TNFα after UCMS and LPS (0.25 μg/kg) exposure may also imply that it didn't result in the clinical situation in which such an increase has been observed [39,40] using much higher concentration of LPS were used (0.25 mg/kg and 0.33 mg/kg respectively). This might as well be related to strain differences or to the fact that UCMS may in fact mimic a sub-nosographic entity in which no such effects are observed. However, the most relevant changes in inflammation may be observed in the brain. Interestingly, the characteristics of brain inflammation do not correlate with the characteristics of inflammation in the periphery, which suggests the differential regulation of both factors. Indeed, microglial activation as a function of UCMS was observed in the infralimbic, medial orbital & cingulate cortices, the amygdala, the hippocampus (CA1) and basal ganglia structures such as the nucleus accumbens and caudate putamen. These findings are in line with previously published data regarding the effects of chronic stress on microglial activity [41,42]. Given the validity, reliability and sensitivity of UCMS model of depression [43,44], this is the strongest evidence of microglial activation as a function of stress reported to date. However, the effects of acute stress on the microglial inflammatory response are still disputed [45,46]. Here, we observed unique characteristics of UCMS-induced microglial activation compared to LPS-induced microglial activation. The UCMS-induced activation of microglia was not different from that induced by intraperitoneal LPS injection. This suggests that UCMS, despite being a combination of mild stressors, may represent an acute inflammatory stimulus. Baseline stress increases the vulnerability of microglia to neuroinflammatory stimuli [47]. While previous reports have used microglial activation as an index of the inflammatory response [48], our results with UCMS suggest that microglia exhibit a ceiling effect of activation after chronic exposure to mild stressors. LPS failed to induce any independent effect on the microglia of mice exposed to UCMS. In fact, it is probable that the UCMS- as well as the LPS-induced effects on microglial activation induce ceiling effects. Therefore, the magnitude of these effects cannot be higher, so that no synergy between both treatments can be observed. As the effects of UCMS or LPS on microglial activation on one side (increase in both case), and on locomotion on the other side (increase for UCMS, decrease for LPS), do not parallel, one can infer that both factors do not correlate. Microglial density varies across brain regions, as does microglial reactivity to different stimuli [49,50]. Recent research has led us to hypothesize that microglia not only defend against neuronal insults but also mediate the neurobiological effects of stress [51,52]. They are neuroprotective initially when they release trophic and anti-inflammatory factors, which enhance cell survival, but they then switch to a pro-inflammatory role. This pro-inflammatory role is characterized by the gradual release of NADPH oxidase and inflammatory cytokines such as IL-1 beta and TNF-alpha. The release of such factors leads to neuronal toxicity [52] (for a review see [53]). UCMS induces a depression-like state, which persists throughout the length of the experiment, starting as early as the second week following the initiation of stress. If seen in conjunction with activated microglia in mice exposed to UCMS, the depression-like state suggests that microglial activation has prevailed long enough to exhibit an inflammatory character. As stated earlier, the chronic nature of microglial activity is the determining factor in neuronal toxicity; exposure to UCMS becomes a primary factor causing chronic neuroinflammation in the mouse brain. It is not clear as yet how neuroinflammation contributes to morphological/anatomical changes in different brain structures in depressed subjects.

UCMS is a validated model of depression. By inducing neuroinflammation in stress-responsive regions of the mouse brain that are similar to the effects of other potentially neuroinflammatory stimuli, this model not only supports the inflammatory hypothesis of depression but also provides further evidence of its strength as an experimental paradigm.

These preliminary results confirm the value of using UCMS to characterize the role of neuroinflammation in the potential acceleration of neurodegenerative disorders as well as the ability

of antidepressant treatments to modulate or prevent this effect. A limitation of this study relates to the fact that we used just one endpoint of depression-like behavior (coat state). Particularly, markers of anhedonia like sucrose-preference test or cookie consumption test would have enabled to associate neuroinflammation with specific behavioral endpoints; further studies are required to address this issue. Treatment focused on the neuroinflammatory component of depressive illness could thus be considered as having therapeutic value in the context of depression. It could serve not only to increase the efficacy and rate of the response to antidepressants among depressed subjects who do not respond to conventional treatment but also to decrease the incidence of neurodegenerative diseases over the long term.

5. Conclusion

UCMS induces chronic neuroinflammation in stress-responsive regions of the mouse brain much like other known stimulators of neuroinflammation, which elucidates the relationship among depressive illness, its underlying inflammatory mechanisms and consequences as well as the possible progression to neurodegeneration.

Conflict of interest

None.

Acknowledgments

Financial assistance from the Higher Education Commission, Islamabad, Pakistan, in the form of a scholarship to Farooq R.K. is gratefully acknowledged. The authors are also very grateful to Petra van Nieuwenhuijzen, Mathieu Nollet, Claire Tronel, Séverine Devers, Maryse Pingaud and Bruno Brizard for their assistance as well as Mme Nadege Brunel (Plateforme Technologique Phenotypage du Petit Animal et Microdosages, Hopital Saint Antoine, Paris, France) for technical assistance with cytokine measurements.

References

[1] Lopez AD, Mathers CD. Measuring the global burden of disease and epidemiological transitions: 2002–2030. Ann Trop Med Parasitol 2006;100:481–99.
[2] Culpepper L. Why do you need to move beyond first-line therapy for major depression? J Clin Psychiatry 2010;71(Suppl. 1):4–9.
[3] Dantzer R, O'Connor JC, Lawson MA, Kelley KW. Inflammation-associated depression: from serotonin to kynurenine. Psychoneuroendocrinology 2011;36:426–36.
[4] Dowlati Y, Herrmann N, Swardfager W, Liu H, Sham L, Reim EK, et al. A meta-analysis of cytokines in major depression. Biol Psychiatry 2010;67:446–57.
[5] Savignac HM, Hyland NP, Dinan TG, Cryan JF. The effects of repeated social interaction stress on behavioural and physiological parameters in a stress-sensitive mouse strain. Behav Brain Res 2011;216:576–84.
[6] Dantzer R. Cytokine, sickness behavior, and depression. Immunol Allergy Clin North Am 2009;29:247–64.
[7] O'Brien SM, Scott LV, Dinan TG. Cytokines: abnormalities in major depression and implications for pharmacological treatment. Hum Psychopharmacol 2004;19:397–403.
[8] Dantzer R, Wollman E, Vitkovic L, Yirmiya R. Cytokines and depression: fortuitous or causative association. Mol Psychiatry 1999;4:328–32.
[9] Gleason OC, Fucci JC, Yates WR, Philipsen MA. Preventing relapse of major depression during interferon-alpha therapy for hepatitis C – a pilot study. Dig Dis Sci 2007;52:2557–63.
[10] Hanisch UK, Kettenmann H. Microglia: active sensor and versatile effector cells in the normal and pathologic brain. Nat Neurosci 2007;10:1387–94.
[11] Alexopoulos GS, Morimoto SS. The inflammation hypothesis in geriatric depression. Int J Geriatr Psychiatry 2011;26:1109–18.
[12] Giulian D, Baker TJ, Shih LC, Lachman LB. Interleukin 1 of the central nervous system is produced by ameboid microglia. J Exp Med 1986;164:594–604.
[13] Tanaka S, Ide M, Shibutani T, Ohtaki H, Numazawa S, Shioda S, et al. Lipopolysaccharide-induced microglial activation induces learning and memory deficits without neuronal cell death in rats. J Neurosci Res 2006;83:557–66.
[14] Yirmiya R, Goshen I. Immune modulation of learning, memory, neural plasticity and neurogenesis. Brain Behav Immun 2011;25:181–213.
[15] Rola R, Mizumatsu S, Otsuka S, Morhardt DR, Noble-Haeusslein LJ, Fishman K, et al. Alterations in hippocampal neurogenesis following traumatic brain injury in mice. Exp Neurol 2006;202:189–99.
[16] Wang Q, Tang XN, Yenari MA. The inflammatory response in stroke. J Neuroimmunol 2007;184:53–68.
[17] Frank-Cannon TC, Alto LT, McAlpine FE, Tansey MG. Does neuroinflammation fan the flame in neurodegenerative diseases. Mol Neurodegener 2009;4:47.
[18] Bentivoglio M, Mariotti R, Bertini G. Neuroinflammation and brain infections: historical context and current perspectives. Brain Res Rev 2011;66:152–73.
[19] Ownby RL, Crocco E, Acevedo A, John V, Loewenstein D. Depression and risk for Alzheimer disease: systematic review, meta-analysis, and metaregression analysis. Arch Gen Psychiatry 2006;63:530–8.
[20] Jacob EL, Gatto NM, Thompson A, Bordelon Y, Ritz B. Occurrence of depression and anxiety prior to Parkinson's disease. Parkinsonism Relat Disord 2010;16:576–81.
[21] Heneka MT, Nadrigny F, Regen T, Martinez-Hernandez A, Dumitrescu-Ozimek L, Terwel D, et al. Locus ceruleus controls Alzheimer's disease pathology by modulating microglial functions through norepinephrine. Proc Natl Acad Sci U S A 2010;107:6058–63.
[22] Chavant F, Deguil J, Pain S, Ingrand I, Milin S, Fauconneau B, et al. Imipramine, in part through tumor necrosis factor alpha inhibition, prevents cognitive decline and beta-amyloid accumulation in a mouse model of Alzheimer's disease. J Pharmacol Exp Ther 2010;332:505–14.
[23] Tanti A, Belzung C. Open questions in current models of antidepressant action. Br J Pharmacol 2010;159:1187–200.
[24] Willner P, Muscat R, Papp M. Chronic mild stress-induced anhedonia: a realistic animal model of depression. Neurosci Biobehav Rev 1992;16:525–34.
[25] Santarelli L, Saxe M, Gross C, Surget A, Battaglia F, Dulawa S, et al. Requirement of hippocampal neurogenesis for the behavioral effects of antidepressants. Science 2003;301:805–9.
[26] Surget A, Saxe M, Leman S, Ibarguen-Vargas Y, Chalon S, Griebel G, et al. Drug-dependent requirement of hippocampal neurogenesis in a model of depression and of antidepressant reversal. Biol Psychiatry 2008;64:293–301.
[27] Isingrini E, Camus V, Le Guisquet AM, Pingaud M, Devers S, Belzung C. Association between repeated unpredictable chronic mild stress (UCMS) procedures with a high fat diet: a model of fluoxetine resistance in mice. PLoS One 2010;5:e10404.
[28] Nollet M, Gaillard P, Minier F, Tanti A, Belzung C, Leman S. Activation of orexin neurons in dorsomedial/perifornical hypothalamus and antidepressant reversal in a rodent model of depression. Neuropharmacology 2011;61:336–46.
[29] Piato AL, Detanico BC, Jesus JF, Lhullier FL, Nunes DS, Elisabetsky E. Effects of Marapuama in the chronic mild stress model: further indication of antidepressant properties. J Ethnopharmacol 2008;118:300–4.
[30] Surget A, Tanti A, Leonardo ED, Laugeray A, Rainer Q, Touma C, et al. Antidepressants recruit new neurons to improve stress response regulation. Mol Psychiatry 2011;16:1177–88.
[31] Nakamura Y, Si QS, Kataoka K. Lipopolysaccharide-induced microglial activation in culture: temporal profiles of morphological change and release of cytokines and nitric oxide. Neurosci Res 1999;35:95–100.
[32] Harada K, Hide I, Seki T, Tanaka S, Nakata Y, Sakai N. Extracellular ATP differentially modulates Toll-like receptor 4-mediated cell survival and death of microglia. J Neurochem 2011;116:1138–47.
[33] Metukuri, Reddy MR, Reddy CM, Reddanna PR. LPS-mediated acute inflammation-induced spermatogenic failure in rats: role of stress response proteins and mitochondrial dysfunction. Inflammation 2010;33:235–43.
[34] Bay-Richter C, Janelidze S, Hallberg L, Brundin L. Changes in behaviour and cytokine expression upon a peripheral immune challenge. Behav Brain Res 2011;222:193–9.
[35] Song C, Wang H. Cytokines mediated inflammation and decreased neurogenesis in animal models of depression. Prog Neuropsychopharmacol Biol Psychiatry 2011;35:760–8.
[36] Liu D, Wang Z, Liu S, Wang F, Zhao S, Hao A. Anti-inflammatory effects of fluoxetine in lipopolysaccharide (LPS)-stimulated microglial cells. Neuropharmacology 2011;61:592–9.
[37] d'Audiffret AC, Frisbee SJ, Stapleton PA, Goodwill AG, Isingrini E, Frisbee JC. Depressive behavior and vascular dysfunction: a link between clinical depression and vascular disease. J Appl Physiol 2010;108:1041–51.
[38] Gibb J, Hayley S, Poulter MO, Anisman H. Effects of stressors and immune activating agents on peripheral and central cytokines in mouse strains that differ in stressor responsivity. Brain Behav Immun 2011;25:468–82.
[39] Godbout JP, Chen J, Abraham J, Richwine AF, Berg BM, Kelley KW, et al. Exaggerated neuroinflammation and sickness behavior in aged mice following activation of the peripheral innate immune system. FASEB J 2005;19:1329–31.
[40] Qin L, Wu X, Block ML, Liu Y, Breese GR, Hong JS, et al. Systemic LPS causes chronic neuroinflammation and progressive neurodegeneration. Glia 2007;55:453–62.
[41] Tynan RJ, Naicker S, Hinwood M, Nalivaiko E, Buller KM, Pow DV, et al. Chronic stress alters the density and morphology of microglia in a subset of stress-responsive brain regions. Brain Behav Immun 2010;24:1058–68.
[42] Wohleb ES, Hanke ML, Corona AW, Powell ND, Stiner LM, Bailey MT, et al. beta-Adrenergic receptor antagonism prevents anxiety-like behavior and microglial reactivity induced by repeated social defeat. J Neurosci 2011;31:6277–88.
[43] Willner P. Validity, reliability and utility of the chronic mild stress model of depression: a 10-year review and evaluation. Psychopharmacology (Berl) 1997;134:319–29.

[44] Willner P. Chronic mild stress (CMS) revisited: consistency and behavioural-neurobiological concordance in the effects of CMS. Neuropsychobiology 2005;52:90–110.
[45] Kwon MS, Seo YJ, Lee JK, Lee HK, Jung JS, Jang JE, et al. The repeated immobilization stress increases IL-1beta immunoreactivities in only neuron, but not astrocyte or microglia in hippocampal CA1 region, striatum and paraventricular nucleus. Neurosci Lett 2008;430:258–63.
[46] Sugama S, Fujita M, Hashimoto M, Conti B. Stress induced morphological microglial activation in the rodent brain: involvement of interleukin-18. Neuroscience 2007;146:1388–99.
[47] de Pablos RM, Villaran RF, Arguelles S, Herrera AJ, Venero JL, Ayala A, et al. Stress increases vulnerability to inflammation in the rat prefrontal cortex. J Neurosci 2006;26:5709–19.
[48] Frank MG, Baratta MV, Sprunger DB, Watkins LR, Maier SF. Microglia serve as a neuroimmune substrate for stress-induced potentiation of CNS pro-inflammatory cytokine responses. Brain Behav Immun 2007;21:47–59.
[49] Pintado C, Revilla E, Vizuete ML, Jimenez S, Garcia-Cuervo L, Vitorica J, et al. Regional difference in inflammatory response to LPS-injection in the brain: role of microglia cell density. J Neuroimmunol 2011;238:44–51.
[50] Lawson LJ, Perry VH, Dri P, Gordon S. Heterogeneity in the distribution and morphology of microglia in the normal adult mouse brain. Neuroscience 1990;39:151–70.
[51] Hinwood M, Morandini J, Day TA, Walker FR., Evidence that microglia mediate the neurobiological effects of chronic psychological stress on the medial prefrontal cortex. Cereb Cortex; 2011, doi:10.1093/cercor/bhr229.
[52] Smith JA, Das A, Ray SK, Banik, NLSmith JA, Das A, Ray SK, Banik NL. Role of pro-inflammatory cytokines released from microglia in neurodegenerative diseases. Brain Res Bull 2012;87:10–20.
[53] Henkel JS, Beers DR, Zhao W, Appel SH. Microglia in ALS: the good, the bad, and the resting. J Neuroimmune Pharmacol 2009;4:389–98.

Effect of P2X7 receptor antagonism on UCMS induced behavioral and biochemical alterations

Depression possesses significant heritability. Various epidemiological studies working on first degree relatives of depression sufferers have reported significantly higher incidence of the disease in monozygotic twins of depressed subjects, dizygotic twins and first degree relatives in the decreasing order. These particular data put emphasis on need to explore genetic basis of this disorder.

Epidemiological studies have also recorded close relationship between single nucleotide polymorphisms (SNPs) of genes that participate in various phenomena related to depressive illness. One such phenomenon is microglial activation which has been established as a major player in the propagation of neuronal injury and depressive and neurodegenerative diseases. It has also been proved to be a part of depressive like syndrome induced by exposure to unpredictable chronic mild stress (UCMS) in mice. Important determinants of microglial activation are pro-inflammatory cytokines IL-1 beta, TNF alpha and IL-6, out of which IL-1 beta has been reported to be the most important one owing to its rate limiting character in induction of antineurogenic and anhedonic effects of stress in mice.

Mechanism of IL-1 liberation in its inactive form and its subsequent activation through post-t6ranslational modification is of immense importance in this process. This process relies on the function of an ATP activated purinergic receptor called P2X7 receptor. Apart from participating in conversion of inactive IL-1 beta to its active form, prolonged activation of this receptor may leads to opening of a transmembranous pore which threatens cellular homeostasis by allowing free movement of high molecular weight moieties. This is the reason this pore is sometimes called the death pore. Second aspect of our concern about role of this receptor in depressive illness comes from single nucleotide polymorphisms of the gene encoding it.

It has been reported that certain SNPs of this gene have been found to be related to incidence of depressive illness. These genetic variations may dictate similar variations in the functioning of the said receptor which may hypothetically be translated into variation of the function of eventual determinants of microglial activation and depressive symptoms related to this activation. This is what prompted us to use an antagonist of P2X7 receptor in UCMS model of depression in a vulnerable strain of mice, first to explore the reality of its involvement and second, to explore its mechanism of participation. We divided mice into two groups, those which were exposed to UCMS and those which were kept in controlled conditions. After two weeks of stress, the two groups of animals were divided into further into three groups each on the basis of the pharmacological treatment initiated, namely a conventional antidepressant (Fluoxetine), a selective antagonist of P2X7 receptor (Brilliant Blue G or BBG) and Vehicle. The evolving effect of UCMS as well as treatment was quantified by coat

state score while nest test was carried out towards the end of protocol.

In order to see UCMS induced changes in HPA axis alterations and effect of pharmacological antagonism of P2X7R on these changes, we performed dexamethasone suppression test. Mice were injected with either vehicle or dexamethasone and mice were subjected to open field exploration half an hour later. Blood was collected two hours following the time of injection and was analyzed by radioimmunoassay to determine the concentration of corticosterone in their blood. Mice brains were collected for immunohistochemistry for doublecortin positive cells.

The results demonstrate that coat state degradation which is a part of depressive like behavior induced by UCMS is reversed by chronic treatment by P2X7 receptor antagonist BBG in the same way as done by an SSRI Fluoxetine. Both of these drugs reversed the impairment of nest building skill in mice which was precipitated by exposure to UCMS. BBG, however, didn't affect number of doublecortin positive cells in stressed mice nor did it in non-stressed mice whereas Fluoxetine caused a significant increase in the same parameter in stressed group. On the contrary, BBG successfully reversed the alteration in the HPA axis which was potentially brought about by exposure to UCMS. These results are significant in a way that they shed light on the mechanism of antidepressant action of pharmacological antagonism of P2X7R. The interesting finding of this study is that this receptor is implicated in induction of UCMS induced depressive like behavior. It is also interesting to note that it exerts its effects through HPA axis and not through modulation of number of new born neurons in the dentate gyrus of mice brain. An estimation of microglial activity related to this intervention will make these mechanisms more clear to understand.

These results are under preparation for submission.

Pharmacological antagonism of P2X7 receptor and reversal of UCMS induced depressive like behavior and HPA axis alterations in mice

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Key words: Neuroinflammation, Depression, UCMS, P2X7 receptor, HPA-axis, Neurogenesis

Abstract

Rising cost of depressive illness in terms of disability and treatment failure is the cause of intense research in this field. Inflammatory factors have shown significant involvement in addition to the previously known monoamine and hormonal alterations in its causation. P2XR7 gene encodes P2X7 receptor protein; its participation in depressive illness has been hypothesized from studies showing an association between single nucleotide polymorphisms of this gene and depression. The mechanism of this involvement is proposed to relate in post-translational modification of IL-1β, a pro-inflammatory cytokine, and subsequent microglial activation. Here we tested the potential effects of its antagonist Brilliant Blue G in unpredictable chronic mild stress (UCMS), an animal model of depression in mice already validated for such studies, by comparing them with an SSRI. Our results indicate that Brilliant Blue G successfully reverses the degradation of coat state and nest building scores brought about by exposure to UCMS, much like the conventional antidepressant Fluoxetine. In contrast to Fluoxetine, it didn't increase density of doublecortin positive cells in dentate gyrus. Instead, it seems to have exerted its antidepressant effect by correcting the deranged hypothalamo-pituitary-adrenal axis regulation which was induced by UCMS as well. These results not only signify the role of P2X7 receptor in the recovery from depressive-like state caused by exposure to UCMS but also shed light on its mechanism of its participation. Future research on these mechanisms can certainly open up new points of interventions for pharmacotherapy of major depression.

1. Introduction

Major depressive disorder is a public health challenge that has been focus of intense research and debate over last few decades. It is projected to become 2nd biggest cause of disability in the world by 2020 (Bakish, D., 2001). The rising cost of illness and disease burden in industrialized world is forcing the funding organizations to dedicate more and more funds for research on pathophysiology of depressive illness (Greenberg, P. E., Kessler, R. C., Birnbaum, H. G. *et al*, 2003;Wittchen, H. U., Jacobi, F., Rehm, J. *et al*, 2011). The main hypotheses of depression pathophysiology i.e. the monoamine and the neuroplastic hypotheses have done well in explaining various phenomena related to its symptomatology but have somehow let the scientists down when it comes to the efficacy of treatment plans based on these hypotheses (Lee, S., Jeong, J., Kwak, Y. *et al*, 2010). The latest addition to the puzzle is the finding of systemic abnormalities associated with depressive illness, its bidirectional relationship with vascular and inflammatory diseases (Das, U. N., 2007). Deranged inflammatory parameters are related to induction of mood symptoms and result in sub-optimal response to treatment. Increased pro-inflammatory cytokines, either resulting from chronic inflammatory disease process or as a result of immunotherapy are implicated in the pathogenesis. Modulation of their receptors as well as variations in the genetic governing them have resulted in significant alterations in response to stress as well as treatment. IL-1β is one of the pro-inflammatory cytokines whose release and maturation is governed by an ATP gated purinergic receptor, called P2X7 receptor. This receptor is expressed on the outer membrane of a heterogeneous population of cells inside and outside of brain including cells of the immune lineage particularly microglia (Visentin, S., Renzi, M., Frank, C. *et al*, 1999). Their expression on microglia is undisputed while its expression on neuronal population it is still being debated (Collo, G., Neidhart, S., Kawashima, E. *et al*, 1997;Puthussery, T. and Fletcher, E. L., 2004). P2X7 receptor belongs to a family of purinergic receptors but possesses some unique characteristics such as a large C terminus (Kaczmarek-Hajek, K., Lorinczi, E., Hausmann, R. *et al*, 2012). The receptor gets activated by increased amount of adenosine triphosphate (ATP) in the surrounding area resulting in a pore formation

which is detrimental to cellular well-being (Sperlagh, B., Vizi, E. S., Wirkner, K. *et al*, 2006;Rassendren, F., Buell, G., Newbolt, A. *et al*, 1997). Owing to its size this pore results in movement of high molecular weight moieties eventually resulting in cell death (Di, Virgilio F., Chiozzi, P., Falzoni, S. *et al*, 1998). It also takes part in post-translational modification of pro-IL-1β into its activated form IL-1β which is the chief pro-inflammatory cytokine in the brain (Ferrari, D., Pizzirani, C., Adinolfi, E. *et al*, 2006). IL-1β deficient mice resisted the trademark anhedonic and antineurogenic effects of chronic stress exposure (Koo, J. W. and Duman, R. S., 2008). Modulation of IL-1β as well as P2X7 receptor function has yielded antidepressant effects in a variety of animal models and situations (Basso, A. M., Bratcher, N. A., Harris, R. R. *et al*, 2009a;Boucher, A. A., Arnold, J. C., Hunt, G. E. *et al*, 2011a;Goshen, I., Kreisel, T., Ben-Menachem-Zidon, O. *et al*, 2008). Single nucleotide polymorphisms of gene encoding P2X7 receptor have been found to be related to depressive illness amongst individuals (Roger, S., Mei, Z. Z., Baldwin, J. M. *et al*, 2010a). The exact mechanism of its involvement in pathogenesis and recovery from depressive, however, remains largely unknown. In the current study, we explored the effects of antagonism of the P2X7 receptor function for its potential outcome in an animal model of depression along with its behavioral and neurobiochemical as well as immunohistochemically determined consequences.

2. Material and Method

2.1. Animals

Six groups of 15 7-week-old male BALB/cByJ@Rj mice (Centre d'élevage JANVIER Le Genest-St-Isle France) were subjected to unpredictable chronic mild stress (UCMS) or housed under standard conditions for 9 weeks. UCMS protocol requires mice to be housed individually while control mice are kept in groups of four or five. Room temperature is strictly controlled (between 22 ±2 ◦C) while light and dark cycle was reversed (lights on from 8:00pm to 8:00am) to allow handling and manipulations to take place during the dark phase of cycle. All procedures (with

obvious exception of overnight nesting test) were carried out in accordance with the veterinary service (agreement number C37-261-2), the Ethics Committee for Animal Experimentation (Val de Loire n◦2011-06-10), the European Community Council directive 86/609/EEC and the Ministry of Agriculture of France. Food and water were provided ad libitum.

2.2. Unpredictable Chronic Mild Stress (UCMS)

UCMS is an animal model of depression in mice which is a variation of chronic mild stress model of depression in rats described by Willner and colleagues (Willner, P., Muscat, R., and Papp, M., 1992). It consists of chronic exposure of a vulnerable strain of mice (BALB/C) to randomized social and environmental stressors of mild to moderate severity over duration of several weeks. Randomization of stressors is done in an order to minimize the predictability of the stressors. Frequently applied stressors include social stresses, wet bedding, and frequent changes of saw dust, tilting of cages at 45 degrees, putting rat droppings in mice cages, playing predator sounds, altering day and night cycles and restraint stress for 30 minutes. Protocol is begun with two stressors daily and is gradually increased to four or five. Food and water deprivation was not used as stressors due to ethical reasons. The evolution of UCMS induced depressive like behavior is done by weekly recording coat state score which is a pharmacologically validated index for this purpose in the said strain of mice (Santarelli, L., Saxe, M., Gross, C. *et al*, 2003;Isingrini, E., Camus, V., Le Guisquet, A. M. *et al*, 2010;Hache, G., Guiard, B. P., Le, Dantec Y. *et al*, 2012). The coat-state score consist of evaluating eight different body parts including head, neck, dorsal coat, ventral coat, tail, forepaws, hind paws and genital region for signs of degradation. A score of 0 was attributed for neat coat and a 1 was given to a dirty one. Sum of these scores was used for statistical analyses Two independent, blinded observers performed evaluation of the coat state and the means of their results were statistically analyzed after testing inter-observer reliability. A general scheme of the protocol followed in current study is given in figure 1.

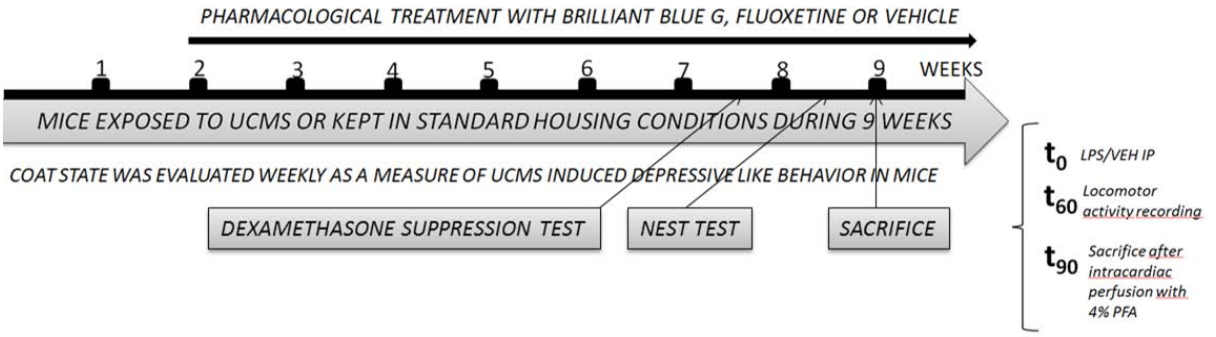

Figure 1: Protocol of unpredictable chronic mild stress. Mice were exposed to UCMS during nine weeks while treatment was started from beginning of third week. Dexamethasone suppression test and nest building test were carried out in 8th and 9th weeks respectively.

2.3. Drugs

Two weeks after initiation of UCMS, each group of mice was divided into three further groups based on intraperitoneal treatment that they received, namely a conventional antidepressant from SSRI group (Fluoxetine @15mg/kg body weight in 0.9% NaCl 10ml/kg/day) (Sequoia, Paris), an antagonist of P2X7 receptor (Brilliant Blue G @ 50mg/kg body weight in 0.9% NaCl 10ml/kg/day) (BioExpress , UT 84037 USA) or Vehicle (NaCl 0.9% 10ml/kg/day). Mice were injected between 12h00 and 14h00 daily. The treatment continued till the end of the protocol and evolution of their effect was calculated by the weekly calculation of coat state score.

2.4. Dexamethasone suppression test

Dexamethasone suppression test was carried out in order to quantify the effect of treatment on UCMS induced alteration of hypothalamo-pituitary-adrenal (HPA) axis regulation. For this purpose, mice were injected with dexamethasone phosphate (D-1756 Sigma-Aldrich) (a glucocorticoid receptor agonist) 0.1mg/kg body weight in 0.9% NaCl 10ml/kg body weight or 0.9% NaCl 10ml/kg body weight only (t_0) only via intraperitoneal route on two different occasions (72 hours apart). Half an hour following the injection, mice were subjected to open field exploration for 5 minutes in order to stimulate the stress axis (t_{30} – t_{35}). Two hours after the

injection (t_{120}), blood was collected from submandibular region in plastic tubes added with the anticoagulant EDTA, centrifuged at 5000 rpm for 10 minutes and plasma was pipetted in a separate tube. The plasma was stored at -80 degree C until defreezed to perform radioimmunoassay for quantification of corticosterone using corticosterone ^{125}I RIA kit (MP Biomedicals NY). A general scheme of the test is given in the figure 2.

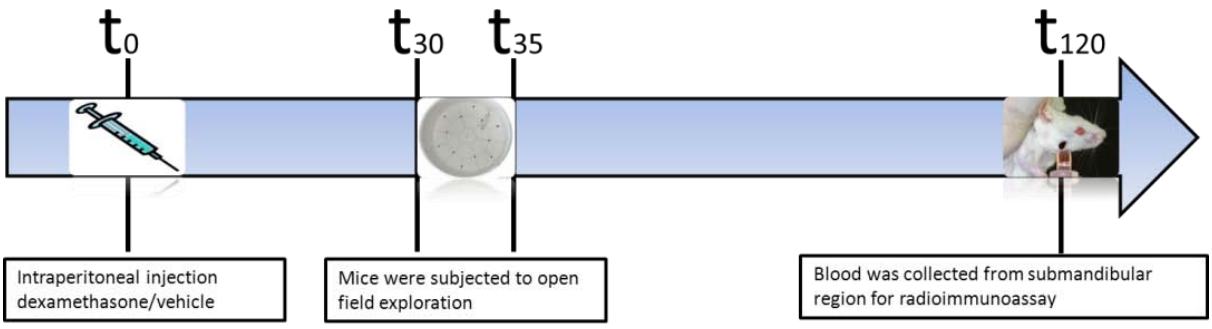

Figure 2: A general scheme of dexamethasone suppression test. Mice were injected with dexamethasone 0.1mg/kg body weight in 0.9% NaCl 10 ml/kg body weight or Saline at t_0. After 30 minutes (t_{30}), they were subjected to open field exploration for five minutes (t_{30} – t_{35}) while blood was collected two hours after injection (t_{120}).

2.5. Nest building test

Nest building test is a measure of integrity of cognition and hippocampal function in mice (Deacon, R. M., Croucher, A., and Rawlins, J. N., 2002). Both control and UCMS mice were transferred to standard cages the evening before the day when the test was scheduled to be performed. A standard piece of cotton 3x3cm was placed in the mouse cages one hour before the commencement of dark phase. First score (score 1) was given 5 hours after putting cotton in the cages while score 2 was given at 24 hours from the time when cotton was placed i.e. one hour before the start of the dark phase on the next morning. The scores were given according to the degree of utilization of cotton and shape of the nest eventually created, as described by Deacon (Deacon, R. M., 2006). The cotton was removed and mice were returned to their respective cages after 2nd score.

2.6. Lipopolysaccharide treatment and recording of locomotor activity

Mice were intraperitoneally injected with bacterial inflammatory antigen (LPS) (Sigma-Aldrich MO 63103 USA), which is a known activator of microglia, at a dose of 0.25 micrograms/kg body weight or vehicle (0.9% saline). An hour later, they were subjected to actimeter equipped with two perpendicular infrared light beams located 1.5 cm above the floor. Mice, in their home cages, were placed in the actimeter and locomotor activity was detected and recorded as number of interruptions of light beams during 30 minutes as a measure of effects LPS induced psychomotor retardation. Transparent cages with 1/3rd of the normal sawdust level were used for this purpose so as to minimize interference with activity detection.

2.7. Sacrifice

Following the recording of locomotor activity, mice were anaesthetized with intraperitoneal injection of pentobarbital (40mg/kg in NaCl 0.9% 10ml/kg body weight). Thorax was cut open and blood was collected from intracardiac puncture, centrifuged at 5000 rpm for 10 minutes and serum was collected for measurement of proinflammatory cytokines. Intracardiac cannula was placed and saline was rushed through the systemic circulation followed by 180 ml of 4% paraformaldehyde for tissue fixation. Mice brain were dissected out and placed in 4% PFA for two hours for post-fixation after which they transferred to 20% sucrose solution and kept in cold room until they were cut in 40micrometer thick slices using cryostat microtome (Leica CM 3050S) and placed in freezing solution at -20 C.

2.8. Analysis of peripheral proinflammatory cytokines

The serum samples were diluted to 1:100 and were quantified by mean of antibody-immobilized beads and Luminex MAP technology (xMAP Luminex IFR65, Paris) in duplicate in the same assay for concentrations of pro-inflammatory cytokines IL-6, IL-1β and tumor necrosis factor alpha using the multiplex immunoassay kit (Millipore Corporate, Billerica, MA).

2.9. Immunohistochemistry for doublecortin

Mice brain slices were rinsed in 0.1M PB for 5 minutes, 50% ethanol for 20 minutes and added with 3% H_2O_2 for further 20 minutes. After washing with 0.1M PB thrice for 10 minutes each, sections were incubated at room temperature in goat anti mouse doublecortin antibodies (Santa-Cruz Biotechnology; diluted 1:500) for 24 h, followed by three washes in PB and a 2-h incubation in secondary donkey anti-goat IgG biotinylated antibody (Jackson Immuno Research, West Grove, Pennsylvania; diluted 1:500). Sections were then incubated for 60 minutes in avidin–biotin–peroxidase complex (Vectastain ABC kit; Vector Laboratories, Burlingame, CA, USA; diluted 1:100) prepared 30 minutes earlier, washed again with PB 0.1M and reacted with freshly prepared diaminobenzidine-HCl (DAB; Sigma–Aldrich, St. Louis, Missouri, USA) in the presence of H_2O_2. The sections were then rinsed in PB and mounted on gelatinized glass slides and kept on room temperature until dried. Slides were then immersed in blue and dehydrated, cleared in Claral (Réactifs RAL) and coverslips (Eukitt®) were applied. The doublecortin positive cells were counted and surface area of dentate gyrus was calculated under the microscope while surface area of dentate gyrus was calculated using Axiovision (Zeiss) software. The values of surface area and number of DCX positive cells were used to calculate density of DCX positive cells in mice brain.

2.10. Statistics

Due to limited number of animals per group, all statistics were carried out using nonparametric tests for analysis of the data related to behavioral as well as biochemical tests performed. When found significant, Kruskal Wallis test was followed by Mann Whitney U test and Friedman test was followed by Wilcoxon test for between the groups comparison when required. All values are expressed as mean +/- SEM. Results were taken as significant when $p<0.05$.

3. Results

3.1. UCMS degrades mice coat state which is reversed by chronic fluoxetine as well as by P2X7 receptor antagonist treatment

The results of UCMS induced depressive like behavior manifested by degradation of coat state score are shown in figure 3. Exposure to UCMS resulted in degradation of the coat state of mice from 2rd week onwards (Group Kruskal-Wallis test 2nd week H (5, N= 80) = 40.56502 p =0.0000, 9th week H (5, N= 78) =51.68103 p =0.0000). The difference between CTRL VEH and UCMS VEH was significant right from the 2nd week of exposure to stress and remained so throughout the protocol (Mann-Whitney U Test 2nd week p<0.000 9th week p<0.000). Difference between UCMS VEH and USMS FLX became apparent from 7th week onwards (Mann-Whitney U Test 7th week p<0.04, 8th week p<0.01, 9th week p<0.005). The UCMS BBG group also showed significant improvement of coat state apparent from markedly different score when compared to UCMS group treated with vehicle (Mann-Whitney U Test 7th week p<0.056, 8th week p<0.000, 9th week p<0.000). The control groups treated with Fluoxetine or Brilliant Blue G showed no degradation of coat state score when compared with controls treated with vehicle.

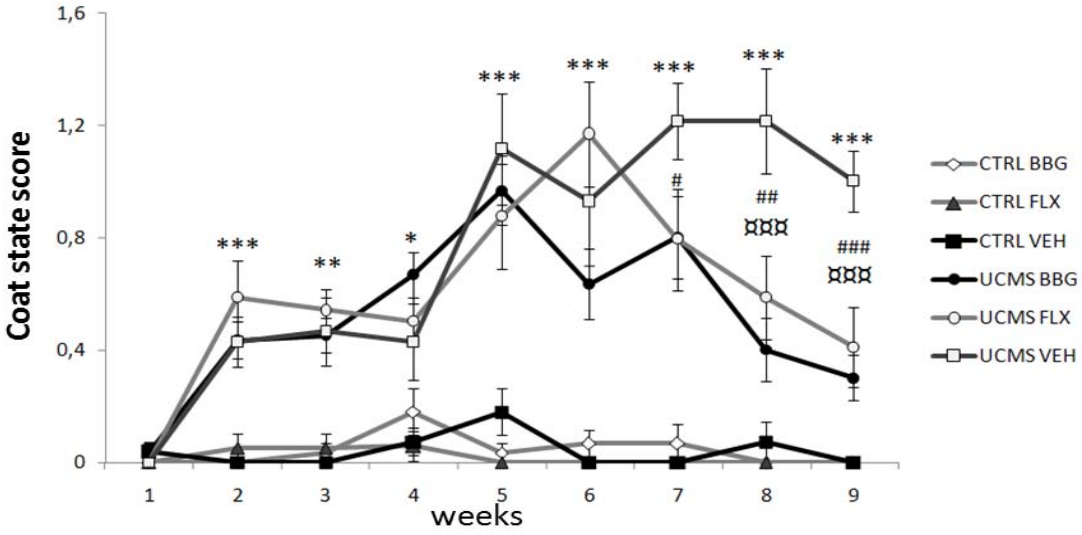

Figure 3: Coat state score showing higher score related to degradation of coat state as a function of exposure to UCMS and its reversal in Fluoxetine (@15mg/kg body weight in

0.9% NaCl 10ml/kg/day) and BBG (@ 50mg/kg body weight in 0.9% NaCl 10ml/kg/day)treated animals while no improvement in those treated with vehicle. Results are presented as mean +/- SEM. * p<0.05 ** p<0.01 *** p<0.001 UCMS VEH vs. CTRL VEH, # p<0.05 ## p<0.01 ### p<0.001, UCMS VEH vs. UCMS FLX, ¤¤¤ p<0.001 UCMS VEH vs. UCMS BBG.

3.2. Intraperitoneal LPS elicits a deficit in locomotor activity and augments peripheral pro-inflammatory cytokine concentrations in mice

LPS injection resulted in reduction of locomotor activity score recorded in 30 minutes in mice in all groups except UCMS FLX group treated with LPS or otherwise (p=0.055) (Group Kruskal-Wallis test: H (11, N= 77) =49.65725 p =0.0000). These results are presented in figure 4. There were no differences of locomotor activity score between different groups as a function of stress or antidepressant and P2X7 receptor antagonist treatment. Peripheral blood was analyzed for concentration of pro-inflammatory cytokine concentration, namely IL-1 (Group Kruskal-Wallis test: H (11, N= 62) =33.51000 p =0.0004), TNFα (Group Kruskal-Wallis test: H (11, N= 55) =29.03718 p =0.0022) and IL-6 (group Kruskal-Wallis test: H (11, N= 76) =45.17677 p =0.0000). The results suggest that UCMS didn't affect any of the three pro-inflammatory cytokines measured neither did Fluoxetine or BBG. Intraperitoneal LPS treatment resulted in increase in the cytokine concentration in all groups when compared to the vehicle treated animals within the respective groups. Individual p values and results in the form of mean +/- SEM are presented in figure 5.

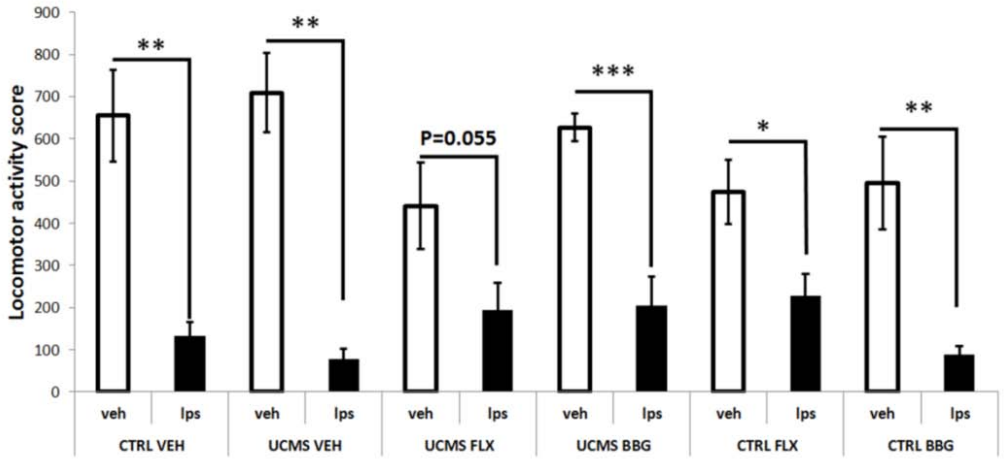

Figure 4: Locomotor activity score showing significant effect of LPS in all but one group of mice. Results are presented as mean +/- SEM. * p<0.05, ** p<0.01 *** p<0.001 vehicle vs. lps.

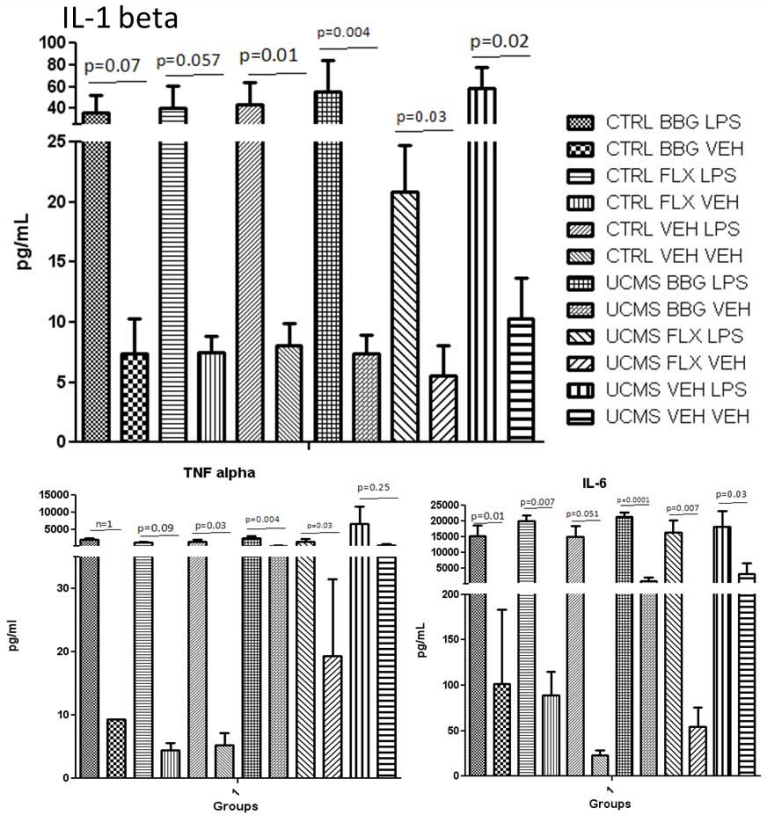

Figure 5: A comparison of pro-inflammatory cytokines IL-1 β, TNF alpha and IL-6. While the effect of LPS is observed in all groups, UCMS didn't elicit a peripheral inflammatory response in mice. Results are presented as mean +/- SEM.

3.3. UCMS induces an impairment of nest making behavior which is reversed by chronic intraperitoneal antidepressant as well as P2X7 receptor antagonist treatment

Nest test score shows that exposure to UCMS resulted in deficit of ability of mice to make nest. The scoring of nests was done 5 and 24 hours after putting the cotton inside mice cages, designated as score 1 and score 2 respectively. Results are presented in Figure 5. Kruskal-Wallis was significant for both score 1 and score 2 (H (5, N= 78) =23.68275 p =0.0002 and H (5, N= 77) =30.80955 p =0.0000 respectively). Score 1 shows significant differences of nest making performance as function of stress as well as Fluoxetine while score 2 shows continuation of markedly poor nest making manifested by the mice that were subjected to UCMS. This ability, however, was re-established in the mice treated with Fluoxetine as well those with BBG over duration of the protocol of stress.

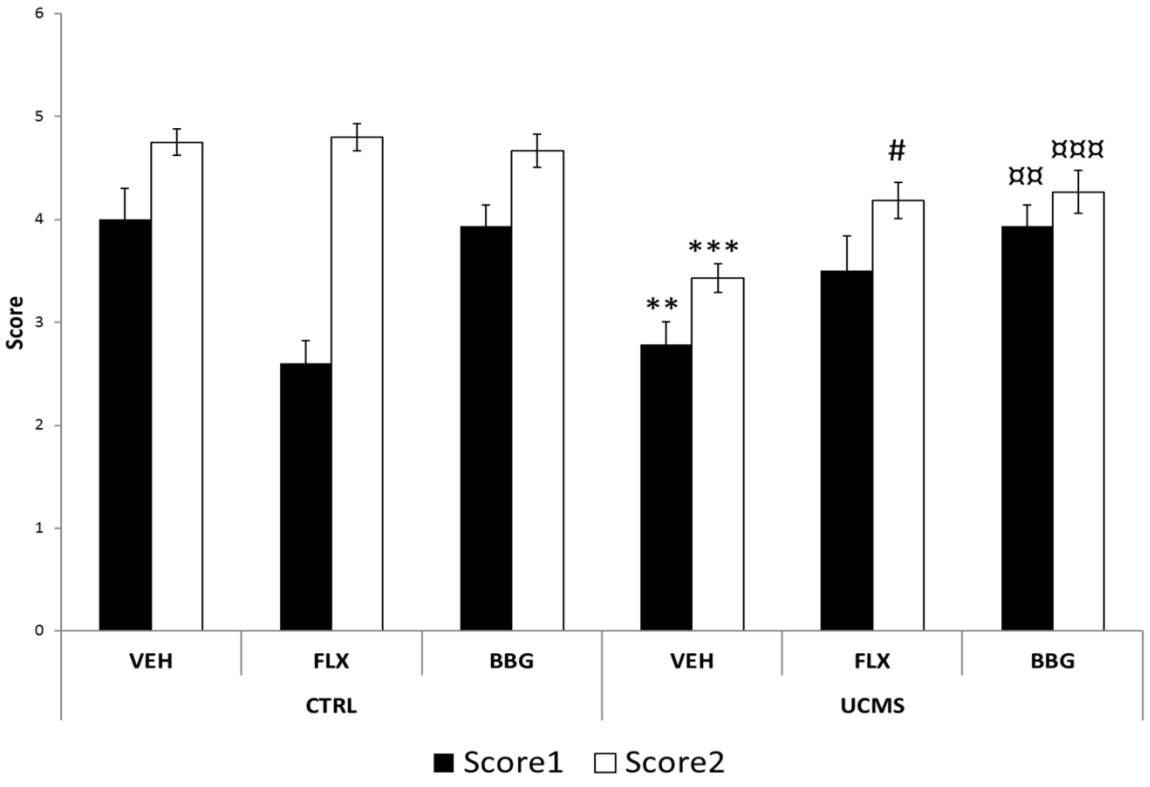

Figure 4: A comparison of nest score amongst different groups of mice. UCMS Fluoxetine as well UCMS BBG mice exhibited a recovery of nest making skills after treatment equal to

controls and in contrast to UCMS Vehicle mice. Results are presented as mean +/- SEM. ** p<0.01 *** p<0.001 CTRL VEH vs. UCMS VEH, # p<0.05 UCMS VEH vs. UCMS FLX, ¤¤ p<0.01 ¤¤¤ p<0.001 UCMS VEH vs. UCMS BBG.

3.4. Chronic Fluoxetine treatment resulted in increased number of doublecortin positive cells

Fluoxetine treatment at the dose of 15mg/kg body weight resulted in significant increase in the number of doublecortin positive cells in dentate gyrus of mice exposed to UCMS (Figure 7). Group Kruskal-Wallis test: H (11, N= 63) =24.05860 p =0.0125. On the contrary, BBG didn't make any impact on this parameter neither in stressed nor non-stressed mice. Fluoxetine didn't have any effect in control mice. The vehicle treated mice were not different between each other as function of exposure to UCMS either.

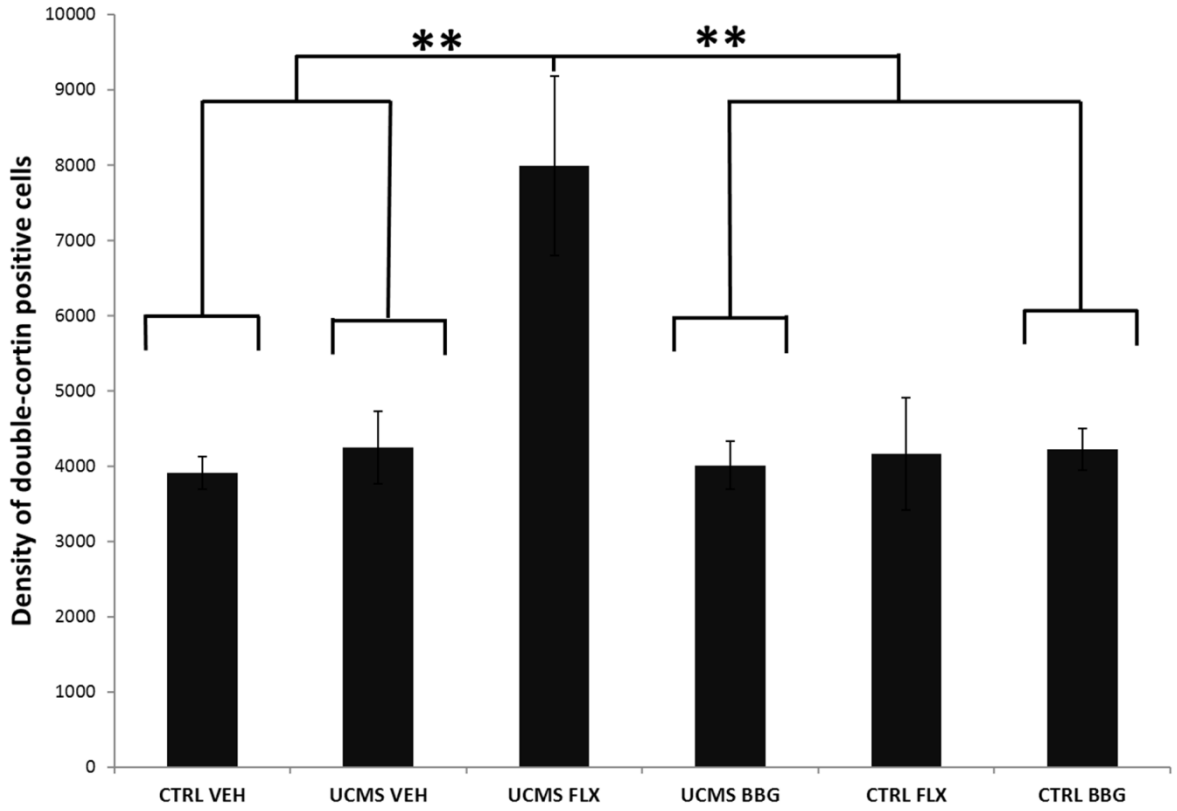

Figure 6: A comparison of density of Doublecortin positive cells in mice dentate gyrus. Only Fluoxetine treated mice showed an increase in density of DCX positive cells when

compared to Control Vehicle, UCMS Vehicle, UCMS treated with BBG or Control BBG. Results are presented as mean +/- SEM. ** p<0.01.

3.5. UCMS results in dysfunction of HPA axis regulation which is effectively reversed by Fluoxetine as well as P2X7 receptor antagonism

Each group of mice was a control for itself for a value of corticosterone after vehicle injection and another following a dexamethasone injection. The suppression of corticosterone concentration following intraperitoneal injection of dexamethasone was found to be significantly perturbed in mice subjected to UCMS while it was remarkably re-established in those treated with Fluoxetine as well as Brilliant Blue G.

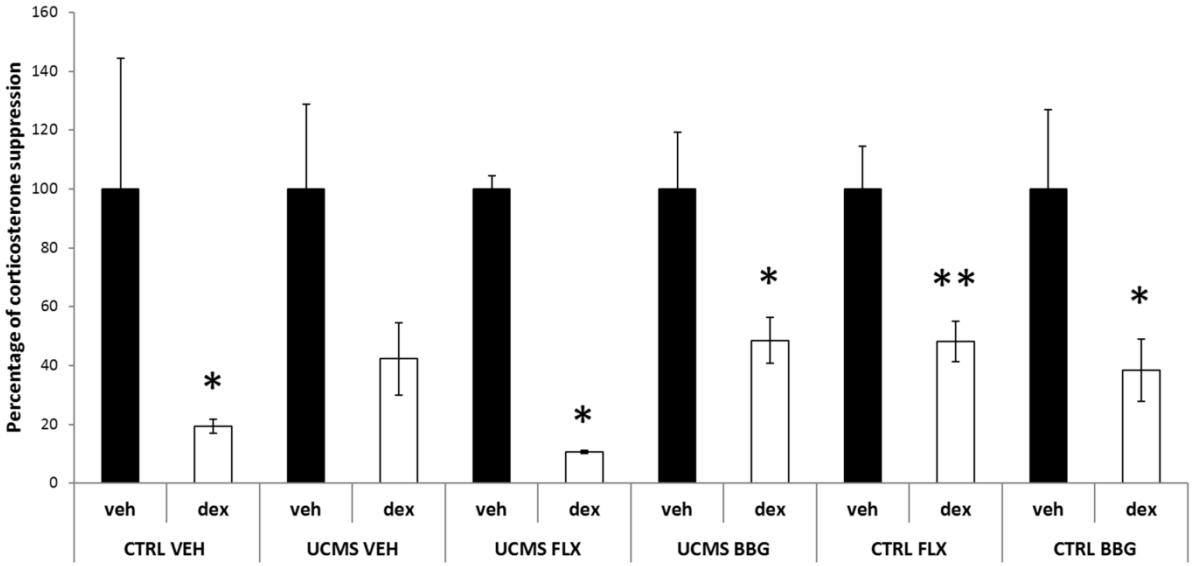

Figure 7: A comparison of corticosterone concentration following intraperitoneal injection of dexamethasone and Vehicle 72 hours apart. The results clearly show that UCMS induced impairment of DEX-suppression and its correction by Fluoxetine and BBG treatment. Results are presented as mean +/- SEM. * p<0.05, ** p<0.01.

4. Discussion

The primary goal of this study was to explore the role of antagonism of P2X7 receptors in recovery from UCMS-induced depressive-like state in mice. UCMS protocol includes exposure of animals to stressors during several weeks and its behavioral and neurobiological effects are measured during as well as after the termination of the protocol. Our results demonstrate a depressive-like behavior in mice as a function of their exposure to UCMS. Coat state and nest test scores were used for this purpose (figure 3 and figure 6 respectively). Depressive like behavior was quantified by weekly evaluation of coat state while nest building capacity of mice was evaluated as a measure of self-oriented behavior. Exposure to UCMS, however, didn't alter concentration of peripheral pro-inflammatory cytokines (Figure 5). These effects were effectively reversed by chronic intraperitoneal treatment with Fluoxetine, an antidepressant from selective serotonin re-uptake inhibitor category. Fluoxetine treatment also resulted in marked increase in the number of double-cortin positive cells in dentate gyrus of mice exposed to UCMS as compared to the mice not treated and/or not exposed to UCMS. However, the number of double-cortin positive cells between vehicle treated control mice and vehicle treated UCMS mice remained unchanged (Figure 7). Fluoxetine also reversed UCMS induced impairment of corticosterone suppression in mice following intraperitoneal injection of dexamethasone (Figure 8). P2X7 receptor antagonist Brilliant Blue G used in parallel with Fluoxetine in this study reversed UCMS induced depressive like state in mice including coat state degradation and impairment of nest making ability of mice exposed to UCMS. It also re-established corticosterone suppression following dexamethasone injection but didn't affect number of double-cortin positive cells in dentate gyrus of mice brain.

The UCMS induced depressive like behavior in mice observed in this study and their reversal by a conventional antidepressant treatment is in accordance with previous studies using the same animal model of depression (Nollet, M., Gaillard, P., Tanti, A. *et al*, 2012;Farooq, R. K., Isingrini, E., Tanti, A. *et al*, 2012;Isingrini, E., Belzung, C., Freslon, J. L. *et al*, 2012;Surget, A., Tanti, A.,

Leonardo, E. D. *et al*, 2011). Lack of a systemic inflammatory reaction as evident by the absence of significant pro-inflammatory cytokine concentration alterations in peripheral blood of mice exposed to UCMS is also in accordance with the our previously published report (Farooq, R. K., Isingrini, E., Tanti, A., Le Guisquet, A. M., Arlicot, N., Minier, F., Leman, S., Chalon, S., Belzung, C., and Camus, V., 2012;Gerber, A. R. and Bale, T. L., 2012).

The notable finding of this study is the reversal of stress induced behavioral and neurobiological effects by chronic intraperitoneal treatment with selective antagonist of P2X7 receptor, Brilliant Blue G (BBG), an antagonist of P2X7 receptor (Jiang, L. H., Mackenzie, A. B., North, R. A. *et al*, 2000), in the UCMS model, in form of reversal of degradation of coat state score, improvement in the functional performance to make a nest and reinstatement of suppression of corticosterone secretion following dexamethasone injection. We also observed that BBG didn't affect the number of doublecortin positive cells in the dentate gyrus of mice as did Fluoxetine. This is an interesting finding in the context of an antidepressant effect which is independent of neurogenesis.

Several studies have implicated modulation of P2X7 receptor function in the recovery from depression. Functional alterations originating from single nucleotide polymorphisms resulting in gain of function have been shown to be related to mood disorders (Barden, N., Harvey, M., Gagne, B. *et al*, 2006a;Lucae, S., Salyakina, D., Barden, N. *et al*, 2006;Roger, S., Mei, Z. Z., Baldwin, J. M. *et al*, 2010b;Barden, N., Harvey, M., Gagne, B. *et al*, 2006b;Lucae, S., Salyakina, D., Barden, N., Harvey, M., Gagne, B., Labbe, M., Binder, E. B., Uhr, M., Paez-Pereda, M., Sillaber, I., Ising, M., Bruckl, T., Lieb, R., Holsboer, F., and Muller-Myhsok, B., 2006). The opposite have been reported using transgenic P2RX7$^{-/-}$ mice, which exhibited an antidepressant profile when tested in different models of repeated stress exposure in animals, namely tail suspension test and forced swim tests in comparison to the wild type ones (Boucher, A. A., Arnold, J. C., Hunt, G. E. *et al*, 2011b). The deletion of P2RX7 gene in mice also resulted in improved response to antidepressant treatment (Basso, A. M., Bratcher, N. A., Harris, R. R. *et al*, 2009b). The

antidepressant phenotype exhibited by transgenic P2RX7$^{-/-}$ mice was reproduced by sub-acute intraperitoneal treatment with P2X7R antagonist BBG in a dose dependent manner (Csolle, C., Ando, R. D., Kittel, A. *et al*, 2012). These studies emphasize on the potential role of P2X7 receptor function modulation in the process of undoing the effects of stress.

Genetic factors do play significant role to predispose subjects to stress induced psychopathologies. They may also hypothesized to be responsible for individual differences in coping skills, threshold of vulnerability as well as gender differences in depression epidemiology (for a review see (Levinson, D. F., 2006). Single nucleotide polymorphisms are associated with alterations in secretion of proinflammatory cytokines and their subsequent contribution to psychopathologies (Clerici, M., Arosio, B., Mundo, E. *et al*, 2009;Illi, J., Miaskowski, C., Cooper, B. *et al*, 2012;Holtzman, S., Abbey, S. E., Chan, C. *et al*, 2012). Genetic variations are also involved in degree of severity of immune response and several other determinants of mood alterations (for a review see (Bufalino, C., Hepgul, N., Aguglia, E. *et al*, 2012). The function of prime pro-inflammatory cytokine IL-1 β has been found to be altered in genetic deletion or experimental antagonism of P2X7 receptor function (Clark, A. K., Staniland, A. A., Marchand, F. *et al*, 2010). The role of Il-1 β has already been established to be indispensable for the anhedonic and antineurogenic effects of stress exposure to take place (Koo, J. W. and Duman, R. S., 2008) which explains the importance of modulation of its determinant factors like P2X7 receptors.

Our results with dexamethasone suppression test also shed light on the possible mechanism of an organism's retrieval from a depressive-like state. Dysregulation of hypothalamo-pituitary-adrenal axis is a hallmark of depressive illness (for a review see (Musselman, D. L. and Nemeroff, C. B., 1996). Acute stress causes an increase in the concentration of corticosteroids (Kudielka, B. M., Buske-Kirschbaum, A., Hellhammer, D. H. *et al*, 2004), which are checked via a complex negative feedback system involving neurotransmitters bringing it to normal levels as soon as the stressor is gone (Carrasco, G. A. and Van de Kar, L. D., 2003). In depressed individuals as a part of pathology, this negative feedback control is typically impaired which leads to elevated

concentrations of corticosteroids for extended periods of time (Holsboer, F. and Barden, N., 1996).

Similar to major depression, UCMS has been showed to evoke dysregulation of HPA axis in mice was reinstated by chronic antidepressant treatment previously (Nollet, M., Gaillard, P., Tanti, A., Girault, V., Belzung, C., and Leman, S., 2012). Current study also reports a UCMS induced impairment of corticosterone suppression following intraperitoneal injection of dexamethasone. Chronic Fluoxetine treatment resulted in re-instatement of this impaired suppression as well as caused an increase in density of double-cortin positive cells in dentate gyrus. BBG, on the other hand, reversed the UCMS-induced corticosterone suppression impairment without altering the number of doublecortin cells in dentate gyrus. These results are noteworthy in view of the role of HPA axis in pathophysiology of major depression. HPA axis alterations are part and parcel of disease process of depressive illness and are frequently predictive of poor response to first line antidepressant treatment (Young, E. A., Altemus, M., Lopez, J. F. *et al*, 2004). It has thus, been suggested that non-responders to conventional antidepressant, which include predominantly those with HPA axis alterations, should be treated with HPA-axis targeted treatment regimens (Holsboer, F., Von, Bardeleben U., Gerken, A. *et al*, 1984). Our results with P2X7 receptor antagonist BBG are remarkable in this regard as we report a reversal of depressive-like state associated with correction of HPA axis alterations but independent of neurogenesis. It may provide a potential point of intervention to improve the percentage of patients benefiting from antidepressant treatment.

Potential limitations of this study are a deficiency of detailed description of behavioral effects of BBG which would have included anhedonic, anxiety and resignation behaviors. Instead of BBG, a more potent and selective antagonist could have used to generate more specific antagonism of P2X7 receptors. Immunohistochemical analysis of microglial activation and receptor expression as well as an analysis of inflammatory cytokine concentration inside the brain structures would generate a more precise picture of the events.

5. Conclusion

Taken together, these results manifest that antagonism of P2X7 receptor can successfully reverse the stress induced depressive-like state as well as the accompanying HPA axis dysregulation. It is also substantial to find neurogenesis independent antidepressant activity of BBG which highlights a potential point of intervention in non-responders, for development of drugs targeting depression related dynamics of HPA-axis directly.

6. Acknowledgements

Higher Education Commission, Islamabad Pakistan provided scholarship to R.K. Farooq which is duly acknowledged. The Authors are also grateful to Anne-Marie Le Guisquet, Mathieu Nollet, Wahid Khemissi and Dorian Sargent for their help. Thanks are also due to MME Nadège Brunel-Meunier and MME Lucette Garreau for providing technical help regarding cytokine and corticosterone dosage respectively.

7. Conflict of interest

None.

8. References

Reference List

1. Bakish, D New standard of depression treatment: remission and full recovery. *J Clin Psychiatry* 2001; **62 Suppl 26:** 5-9.

2. Barden, N, Harvey, M, Gagne, B, Shink, E, Tremblay, M, Raymond, C, Labbe, M, Villeneuve, A, Rochette, D, Bordeleau, L, Stadler, H, Holsboer, F, Muller-Myhsok, B Analysis of single nucleotide polymorphisms in genes in the chromosome 12Q24.31 region points to P2RX7 as a susceptibility gene to bipolar affective disorder. *Am J Med Genet B Neuropsychiatr Genet* 2006a; **141B:** 374-82.

3. Barden, N, Harvey, M, Gagne, B, Shink, E, Tremblay, M, Raymond, C, Labbe, M, Villeneuve, A, Rochette, D, Bordeleau, L, Stadler, H, Holsboer, F, Muller-Myhsok, B Analysis of single nucleotide polymorphisms in genes in the chromosome 12Q24.31 region points to P2RX7 as

a susceptibility gene to bipolar affective disorder. *Am J Med Genet B Neuropsychiatr Genet* 2006b; **141B:** 374-82.

4. Basso, AM, Bratcher, NA, Harris, RR, Jarvis, MF, Decker, MW, Rueter, LE Behavioral profile of P2X7 receptor knockout mice in animal models of depression and anxiety: relevance for neuropsychiatric disorders. *Behav Brain Res* 2009a; **198:** 83-90.

5. Basso, AM, Bratcher, NA, Harris, RR, Jarvis, MF, Decker, MW, Rueter, LE Behavioral profile of P2X7 receptor knockout mice in animal models of depression and anxiety: relevance for neuropsychiatric disorders. *Behav Brain Res* 2009b; **198:** 83-90.

6. Boucher, AA, Arnold, JC, Hunt, GE, Spiro, A, Spencer, J, Brown, C, McGregor, IS, Bennett, MR, Kassiou, M Resilience and reduced c-Fos expression in P2X7 receptor knockout mice exposed to repeated forced swim test. *Neuroscience* 2011a; **189:** 170-177.

7. Boucher, AA, Arnold, JC, Hunt, GE, Spiro, A, Spencer, J, Brown, C, McGregor, IS, Bennett, MR, Kassiou, M Resilience and reduced c-Fos expression in P2X7 receptor knockout mice exposed to repeated forced swim test. *Neuroscience* 2011b; **189:** 170-177.

8. Bufalino, C, Hepgul, N, Aguglia, E, Pariante, CM The role of immune genes in the association between depression and inflammation: A review of recent clinical studies. *Brain Behav Immun* 2012.

9. Carrasco, GA, Van de Kar, LD Neuroendocrine pharmacology of stress. *Eur J Pharmacol* 2003; **463:** 235-72.

10. Clark, AK, Staniland, AA, Marchand, F, Kaan, TK, McMahon, SB, Malcangio, M P2X7-dependent release of interleukin-1β and nociception in the spinal cord following lipopolysaccharide. *J Neurosci* 2010; **30:** 573-82.

11. Clerici, M, Arosio, B, Mundo, E, Cattaneo, E, Pozzoli, S, Dell'osso, B, Vergani, C, Trabattoni, D, Altamura, AC Cytokine polymorphisms in the pathophysiology of mood disorders. *CNS Spectr* 2009; **14:** 419-25.

12. Collo, G, Neidhart, S, Kawashima, E, Kosco-Vilbois, M, North, RA, Buell, G Tissue distribution of the P2X7 receptor. *Neuropharmacology* 1997; **36:** 1277-83.

13. Csolle, C, Ando, RD, Kittel, A, Goloncser, F, Baranyi, M, Soproni, K, Zelena, D, Haller, J, Nemeth, T, Mocsai, A, Sperlagh, B The absence of P2X7 receptors (P2rx7) on non-haematopoietic cells leads to selective alteration in mood-related behaviour with dysregulated gene expression and stress reactivity in mice. *Int J Neuropsychopharmacol* 2012; 1-21.

14. Das, UN Is depression a low-grade systemic inflammatory condition? *Am J Clin Nutr* 2007; **85:** 1665-66.

15. Deacon, RM Assessing nest building in mice. *Nat Protoc* 2006; **1:** 1117-19.

16. Deacon, RM, Croucher, A, Rawlins, JN Hippocampal cytotoxic lesion effects on species-typical behaviours in mice. *Behav Brain Res* 2002; **132:** 203-13.

17. Di, VF, Chiozzi, P, Falzoni, S, Ferrari, D, Sanz, JM, Venketaraman, V, Baricordi, OR Cytolytic P2X purinoceptors. *Cell Death Differ* 1998; **5:** 191-99.

18. Farooq, RK, Isingrini, E, Tanti, A, Le Guisquet, AM, Arlicot, N, Minier, F, Leman, S, Chalon, S, Belzung, C, Camus, V Is unpredictable chronic mild stress (UCMS) a reliable model to study depression-induced neuroinflammation? *Behav Brain Res* 2012; **231:** 130-137.

19. Ferrari, D, Pizzirani, C, Adinolfi, E, Lemoli, RM, Curti, A, Idzko, M, Panther, E, Di, VF The P2X7 receptor: a key player in IL-1 processing and release. *J Immunol* 2006; **176:** 3877-83.

20. Gerber, AR, Bale, TL Antiinflammatory Treatment Ameliorates HPA Stress Axis Dysfunction in a Mouse Model of Stress Sensitivity. *Endocrinology* 2012.

21. Goshen, I, Kreisel, T, Ben-Menachem-Zidon, O, Licht, T, Weidenfeld, J, Ben-Hur, T, Yirmiya, R Brain interleukin-1 mediates chronic stress-induced depression in mice via adrenocortical activation and hippocampal neurogenesis suppression. *Mol Psychiatry* 2008; **13:** 717-28.

22. Greenberg, PE, Kessler, RC, Birnbaum, HG, Leong, SA, Lowe, SW, Berglund, PA, Corey-Lisle, PK The economic burden of depression in the United States: how did it change between 1990 and 2000? *J Clin Psychiatry* 2003; **64:** 1465-75.

23. Hache, G, Guiard, BP, Le, DY, Orvoen, S, David, DJ, Gardier, AM, Coudore, F Antinociceptive effects of fluoxetine in a mouse model of anxiety/depression. *Neuroreport* 2012; **23:** 525-29.

24. Holsboer, F, Barden, N Antidepressants and hypothalamic-pituitary-adrenocortical regulation. *Endocr Rev* 1996; **17:** 187-205.

25. Holsboer, F, Von, BU, Gerken, A, Stalla, GK, Muller, OA Blunted corticotropin and normal cortisol response to human corticotropin-releasing factor in depression. *N Engl J Med* 1984; **311:** 1127.

26. Holtzman, S, Abbey, SE, Chan, C, Bargman, JM, Stewart, DE A genetic predisposition to produce low levels of IL-10 is related to depressive symptoms: a pilot study of patients with end stage renal disease. *Psychosomatics* 2012; **53:** 155-61.

27. Illi, J, Miaskowski, C, Cooper, B, Levine, JD, Dunn, L, West, C, Dodd, M, Dhruva, A, Paul, SM, Baggott, C, Cataldo, J, Langford, D, Schmidt, B, Aouizerat, BE Association between pro- and anti-inflammatory cytokine genes and a symptom cluster of pain, fatigue, sleep disturbance, and depression. *Cytokine* 2012; **58:** 437-47.

28. Isingrini, E, Belzung, C, Freslon, JL, Machet, MC, Camus, V Fluoxetine effect on aortic nitric oxide-dependent vasorelaxation in the unpredictable chronic mild stress model of depression in mice. *Psychosom Med* 2012; **74:** 63-72.

29. Isingrini, E, Camus, V, Le Guisquet, AM, Pingaud, M, Devers, S, Belzung, C Association between repeated unpredictable chronic mild stress (UCMS) procedures with a high fat diet: a model of fluoxetine resistance in mice. *PLoS One* 2010; **5:** e10404.

30. Jiang, LH, Mackenzie, AB, North, RA, Surprenant, A Brilliant blue G selectively blocks ATP-gated rat P2X(7) receptors. *Mol Pharmacol* 2000; **58:** 82-88.

31. Kaczmarek-Hajek, K, Lorinczi, E, Hausmann, R, Nicke, A Molecular and functional properties of P2X receptors--recent progress and persisting challenges. *Purinergic Signal* 2012; **8:** 375-417.

32. Koo, JW, Duman, RS IL-1β is an essential mediator of the antineurogenic and anhedonic effects of stress. *Proc Natl Acad Sci U S A* 2008; **105:** 751-56.

33. Kudielka, BM, Buske-Kirschbaum, A, Hellhammer, DH, Kirschbaum, C HPA axis responses to laboratory psychosocial stress in healthy elderly adults, younger adults, and children: impact of age and gender. *Psychoneuroendocrinology* 2004; **29:** 83-98.

34. Lee, S, Jeong, J, Kwak, Y, Park, SK Depression research: where are we now? *Mol Brain* 2010; **3:** 8.

35. Levinson, DF The genetics of depression: a review. *Biol Psychiatry* 2006; **60:** 84-92.

36. Lucae, S, Salyakina, D, Barden, N, Harvey, M, Gagne, B, Labbe, M, Binder, EB, Uhr, M, Paez-Pereda, M, Sillaber, I, Ising, M, Bruckl, T, Lieb, R, Holsboer, F, Muller-Myhsok, B P2RX7, a gene coding for a purinergic ligand-gated ion channel, is associated with major depressive disorder. *Hum Mol Genet* 2006; **15:** 2438-45.

37. Musselman, DL, Nemeroff, CB Depression and endocrine disorders: focus on the thyroid and adrenal system. *Br J Psychiatry Suppl* 1996; 123-28.

38. Nollet, M, Gaillard, P, Tanti, A, Girault, V, Belzung, C, Leman, S Neurogenesis-independent antidepressant-like effects on behavior and stress axis response of a dual orexin receptor antagonist in a rodent model of depression. *Neuropsychopharmacology* 2012; **37:** 2210-2221.

39. Puthussery, T, Fletcher, EL Synaptic localization of P2X7 receptors in the rat retina. *J Comp Neurol* 2004; **472:** 13-23.

40. Rassendren, F, Buell, G, Newbolt, A, North, RA, Surprenant, A Identification of amino acid residues contributing to the pore of a P2X receptor. *EMBO J* 1997; **16:** 3446-54.

41. Roger, S, Mei, ZZ, Baldwin, JM, Dong, L, Bradley, H, Baldwin, SA, Surprenant, A, Jiang, LH Single nucleotide polymorphisms that were identified in affective mood disorders affect ATP-activated P2X7 receptor functions. *J Psychiatr Res* 2010a; **44:** 347-55.

42. Roger, S, Mei, ZZ, Baldwin, JM, Dong, L, Bradley, H, Baldwin, SA, Surprenant, A, Jiang, LH Single nucleotide polymorphisms that were identified in affective mood disorders affect ATP-activated P2X7 receptor functions. *J Psychiatr Res* 2010b; **44:** 347-55.

43. Santarelli, L, Saxe, M, Gross, C, Surget, A, Battaglia, F, Dulawa, S, Weisstaub, N, Lee, J, Duman, R, Arancio, O, Belzung, C, Hen, R Requirement of hippocampal neurogenesis for the behavioral effects of antidepressants. *Science* 2003; **301:** 805-9.

44. Sperlagh, B, Vizi, ES, Wirkner, K, Illes, P P2X7 receptors in the nervous system. *Prog Neurobiol* 2006; **78:** 327-46.

45. Surget, A, Tanti, A, Leonardo, ED, Laugeray, A, Rainer, Q, Touma, C, Palme, R, Griebel, G, Ibarguen-Vargas, Y, Hen, R, Belzung, C Antidepressants recruit new neurons to improve stress response regulation. *Mol Psychiatry* 2011; **16:** 1177-88.

46. Visentin, S, Renzi, M, Frank, C, Greco, A, Levi, G Two different ionotropic receptors are activated by ATP in rat microglia. *J Physiol* 1999; **519 Pt 3:** 723-36.

47. Willner, P, Muscat, R, Papp, M Chronic mild stress-induced anhedonia: a realistic animal model of depression. *Neurosci Biobehav Rev* 1992; **16:** 525-34.

48. Wittchen, HU, Jacobi, F, Rehm, J, Gustavsson, A, Svensson, M, Jonsson, B, Olesen, J, Allgulander, C, Alonso, J, Faravelli, C, Fratiglioni, L, Jennum, P, Lieb, R, Maercker, A, van, OJ, Preisig, M, Salvador-Carulla, L, Simon, R, Steinhausen, HC The size and burden of mental disorders and other disorders of the brain in Europe 2010. *Eur Neuropsychopharmacol* 2011; **21:** 655-79.

49. Young, EA, Altemus, M, Lopez, JF, Kocsis, JH, Schatzberg, AF, DeBattista, C, Zubieta, JK HPA axis activation in major depression and response to fluoxetine: a pilot study. *Psychoneuroendocrinology* 2004; **29:** 1198-204.

Discussion

Discussion

The objective of this thesis was to investigate the dynamics of neuroinflammatory component of UCMS induced depressive like behavior in mice by exploring the role and mechanism of contribution of P2X7 receptor function. Given the association of depressive symptoms with chronic inflammatory diseases and cytokine immunotherapy, we looked for central as well as peripheral aspects of the inflammatory features of depressive like behavior. In view of the important role in liberation and maturation of IL-1β played by P2X7 receptors, it was hypothesized that an antagonism of this receptor will potentially help undo the effects of stress which are chiefly mediated by IL-1β. Our pursuit of finding the mechanism of interaction between depressive symptoms and inflammatory determinants has been fruitful given the scope of our results. The important aspects of our results are the association of microglial activation with the UCMS-induced depressive like behavior and the evidence that as a function of exposure to mild stressors, this inflammatory character is confined to brain and periphery is spared of such fluctuations. Choice of the animal model of depression used in this project as well as significance of these results and their perspective applications are discussed in the following sections.

7.1. UCMS as a model to studying neuroinflammation

In a bid to elaborate the co-existence of neuroinflammation and depression, we carried out a validation study for the UCMS model of depression for this parameter. It was aimed to see the effects of UCMS on central and peripheral inflammatory parameters in mice. To our knowledge none of these factors had been tested using this mice model of depression previously. Following the protocol of UCMS, mice were injected with LPS which is widely used as an immune challenge and is a known activator of microglia (Nakamura, Y., Si, Q. S., and Kataoka, K., 1999) in order to generate microglial activation to make a comparison with the effects of UCMS. On a behavioral level, UCMS induced a depressive-like state in mice which was evaluated from degradation or tidiness of their coat state, a pharmacologically validated index as a marker of depressive like behavior in this strain of mice (Isingrini, E., Camus, V., Le Guisquet, A. M. *et al*, 2010a). Pro-inflammatory cytokines were measured in the peripheral blood while immunohistochemistry using anti CD11b antibodies

was performed to quantify microglial activation. Both LPS and UCMS increased microglial activation compared to control in various parts of mice brain including cortex (infralimbic, medial orbital, cingulate), brain nuclei (nucleus accumbens, caudate putamen and amygdala) and CA1 region of hippocampus. No synergistic effect of the two factors was observed on microglial activation though. Surprisingly, UCMS didn't alter the peripheral cytokine concentration. These results validated the idea that neuroinflammation occurs as a part of UCMS-induced depressive like behavior. It also encouraged us to proceed with our original hypothesis, which was to explore role of P2X7 receptors in induction of this phenomenon. These results were published in Behavioral Brain Research and have been presented in *Section 5: Results* of this thesis.

It is important to note that UCMS induced neuroinflammatory state has its typical characteristics which may be different from those observed with the use of acute or relatively severer stressors (Fatouros, I., Chatzinikolaou, A., Paltoglou, G. *et al*, 2010). These differences can be observed in terms of limitations of eventual inflammatory state as well as characteristics of the components of the inflammatory change itself. It has been noted that chronic stress alters the morphology of microglia by increasing ramifications but doesn't express the markers of cellular injury (Hinwood, M., Tynan, R. J., Charnley, J. L. *et al*, 2012). As shown in figure 14 these morphological changes have been characterized by large cell bodies and retracted processes as compared to appearance of cells from control animals (Diz-Chaves, Y., Pernia, O., Carrero, P. *et al*, 2012). In our studies, we tried to stain microglia with anti CD68 antibodies which is a marker of cellular injury. UCMS induced microglia, however, didn't result in expression of CD68 antigen. So we chose anti CD11b antibodies and calculated the surface area of the cells using histolab software taking it as a marker of increase in the activity of the cells under study. The absence of inflammatory alterations in the periphery indicates another difference that mild stressors exercise when applied chronically. The finding this validation study has important implications for this as well as future projects to be carried out using this model. Evidence of induction of microglial activation is an important character of an animal model of

depression for validation and acceptance purposes.

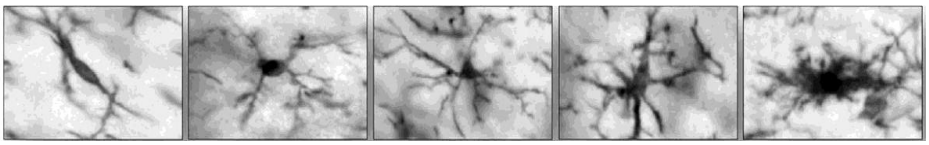

Figure 12: Different morphological appearances of microglia in the dentate gyrus of mice exposed to prenatal stress (Diz-Chaves, Y., Pernia, O., Carrero, P., and Garcia-Segura, L. M., 2012).

Several animal models of depression using variety of animals (rodents, primates) and stress regimes (acute, chronic, mild and severe) have been proposed. They range from an immune challenge resulting in depressive-like behavior in mice (Bay-Richter, C., Janelidze, S., Hallberg, L. *et al*, 2011) to exposure of a suitable species of animals to different types of stressors for shorter as well as longer duration of time (Strekalova, T. and Steinbusch, H. W., 2010;Mineur, Y. S., Belzung, C., and Crusio, W. E., 2006;Yalcin, I., Belzung, C., and Surget, A., 2008a). Choice of animal models largely depends upon scientific reasons (response to stress, comprehension of behavior and cognition of the animal, response to pharmacotherapy) and logistic reasons (ease of handling and housing, availability of the expert manpower). Identifying a vulnerable species is an important part of the construction of a model of depression. It undertakes the fact that only vulnerable people among us tend to develop symptoms of a disease when faced by physiological or psychological stressors which are well-handled by a lot of others without any potential symptomatology (Zozulya, A. A., Gabaeva, M. V., Sokolov, O. Y. *et al*, 2008).

These models are judged on the basis of their ability to fulfill certain criteria of validity. Different authors have proposed different criteria for the validation of animal models of depression. Take for instance the three validation criteria of Willner (Willner, P., 1986). He proposed that an animal model of depression should be judged on its ability to fulfill the face validity, construct validity and predictive validity. Depression, in simple terms, is a disease that occurs to a person who is confronted by a stress, is characterized by lowering of mood and feeling of helplessness and worthlessness among other symptoms which occur through decrease in neurogenesis, alterations in stress hormone axis and amine metabolism

perturbations. With the available treatments, it is possible to achieve a remission when administered on chronic basis and not for short periods of time. An animal model would be validated if it involves precipitation of depressive-like symptoms as a function of exposure to stress occurring through hormonal and neurogenic alterations and which is reversible through chronic treatment with antidepressants. UCMS, the model used in course of this thesis, has earned much respect when we discuss its ability to fulfill the above mentioned criteria (for a review see (Willner, P., 1997). The strain of mice selected for this protocol (BALB/C) as well the antidepressant and its route and dose applied (Fluoxetine, intraperitoneal, @15mg/kg/day) are previously validated using same stressors (Isingrini, E., Surget, A., Belzung, C. *et al*, 2011;Yalcin, I., Belzung, C., and Surget, A., 2008b).

More recently, Belzung and Lemoine have proposed new criteria for evaluation of an animal model. They have stated as much as 9 criteria with elaboration of those stated by the Willner previously and also including new ones *(Figure 7, section 1.7.2 Validation criteria of Belzung and Lemoine)*. Again, UCMS has been able to make it up to the top with maximum of the features of major depressive disorder. Some of the outstanding findings using this model have been the demonstration of depressive like behavior and resignation behavior accompanied by the clinically encountered vascular abnormalities and their proposed mechanism of co-prevalence (Isingrini, E., Belzung, C., d'Audiffret, A. *et al*, 2011a;Isingrini, E., Camus, V., Le Guisquet, A. M. *et al*, 2010b). The hallmark findings of depression such as decrease in neurogenesis as well as alterations in the HPA axis have also been manifested by UCMS exposure (Nollet, M., Gaillard, P., Tanti, A. *et al*, 2012;Surget, A., Tanti, A., Leonardo, E. D. *et al*, 2011). It was, thus, pertinent to see the HPA-axis alterations in view of our pursuit to explore the underlying mechanism of action of neuroinflammation which was done using dexamethasone suppression test, described in the next section.

Preclinical research has reported significant evidence for presence of inflammation as a part of depressive illness, be it in the form of microglial activation or increased expression of pro-inflammatory cytokine markers

(Tynan, R. J., Naicker, S., Hinwood, M. *et al*, 2010;Shimoda, M., Jones, V. C., Kobayashi, M. *et al*, 2006;Hinwood, M., Tynan, R. J., Charnley, J. L., Beynon, S. B., Day, T. A., and Walker, F. R., 2012) and for a review see (Beumer, W., Gibney, S. M., Drexhage, R. C. *et al*, 2012). Human studies have also reported valuable evidence regarding the over-expression of inflammatory markers in depressed subjects (Steiner, J., Walter, M., Gos, T. *et al*, 2011;Kaestner, F., Hettich, M., Peters, M. *et al*, 2005;Miller, A. H., Maletic, V., and Raison, C. L., 2009;Zorrilla, E. P., Luborsky, L., McKay, J. R. *et al*, 2001;Seidel, A., Arolt, V., Hunstiger, M. *et al*, 1996). The valuable information that our results add to the pre-existing understanding of the subject is the evidence of presence of microglial activity inside the brain without any inflammatory alteration in the periphery, in addition to the validation of the UCMS model to study this phenomenon. The absence of peripheral inflammatory alterations is attributed to the severity of stressors applied as they are proportionate to the severity of the symptoms in a given episode of depression (Suarez, E. C., Krishnan, R. R., and Lewis, J. G., 2003).

7.2. Pharmacological antagonism of P2X7Rs in UCMS

Encouraged by the findings of the validation study, we explored the possibility of using an antagonist of P2X7 receptor (Brilliant Blue G or BBG) for its potential antidepressant effects in comparison with a conventional antidepressant from SSRI group (Fluoxetine in this case). The alleged role of P2X7Rs in depression via their participation in liberation and maturation of IL-1β have already been elaborated in introduction part *(Section 3.3.1 and 3.3.2)*. Their antagonism was expected to help reverse the process of neuroinflammation as well as recovery from behavioral deficit induced by UCMS by a possible modulation of IL-1β release and post-translational processing. Mice were subjected to UCMS or kept in control conditions and intraperitoneal treatment with a conventional antidepressant from SSRI group (Fluoxetine15mg/kg/day), P2X7 receptor antagonist (BBG 50mg/kg/day) or Vehicle (NaCl 0.9%) was started from 3rd week onwards. Depressive like behavior was quantified by weekly coat state score and nest test. Other parameters tested included dexamethasone suppression test for corticosterone, peripheral blood for measurement of pro-inflammatory cytokines and density of double-cortin positive cells in dentate gyrus as a marker of neurogenesis. Fluoxetine and BBG both

resulted in reversal of depressive like behavior in mice exposed to stress yet there was a difference between their effects on neuroendocrine and neurogenic components of their effects. Fluoxetine resulted in an increase in density of double-cortin positive cells as well as reversal of impairment of corticosterone secretion suppression following dexamethasone injection while BBG only re-established the perturbation of the impairment of corticosterone suppression and didn't alter number of double-cortin positive cells neither in stressed nor in control mice. In the periphery, like our first experiment, no alteration of pro-inflammatory cytokine was observed.

Our choice of the behavioral parameters was based on the previous experiments carried out using the same model of depression. We have reported a degradation of coat state as function of stress in several experiments in this particular strain of mice (Isingrini, E., Belzung, C., d'Audiffret, A. *et al*, 2011b;Isingrini, E., Camus, V., Le Guisquet, A. M. *et al*, 2010c;Ibarguen-Vargas, Y., Surget, A., Touma, C. *et al*, 2008;Yalcin, I., Coubard, S., Bodard, S. *et al*, 2008;Yalcin, I., Belzung, C., and Surget, A., 2008c;Surget, A., Wang, Y., Leman, S. *et al*, 2009;Surget, A., Saxe, M., Leman, S. *et al*, 2008). Nest building test has also been reported to be a measure of normal behavior of mice and is disrupted in face of inflammatory insults and stress (Chlodzinska, N., Gajerska, M., Bartkowska, K. *et al*, 2011;Hess, S. E., Rohr, S., Dufour, B. D. *et al*, 2008).

Behavioral modifications have already been reported when P2X7 receptor function is altered (Csolle, C., Ando, R. D., Kittel, A. *et al*, 2012). Knockout models as well as antagonism of this receptor have been associated with different mood phenotypes in different situations (Basso, A. M., Bratcher, N. A., Harris, R. R. *et al*, 2009;Csolle, C., Ando, R. D., Kittel, A., Goloncser, F., Baranyi, M., Soproni, K., Zelena, D., Haller, J., Nemeth, T., Mocsai, A., and Sperlagh, B., 2012). Regarding mechanism of its action, direct involvement in the alterations of immune system especially IL-1β and c-fos expression has been reported (Labrousse, V. F., Costes, L., Aubert, A. *et al*, 2009). Microglia activation and IL-1β have been implicated in the behavioral outcome in our first experiment as well elsewhere (Tanaka, S., Ide, M., Shibutani, T. *et al*, 2006;Koo, J. W. and Duman, R. S., 2008;Farooq, R. K.,

Isingrini, E., Tanti, A. *et al*, 2012). Despite this treasured evidence, mechanism of implication P2X7 receptors in psychopathology or recovery has not been clear so far.

Both HPA axis and immune system of an individual are involved in stressful condition and the subject's adaptive response to stress. Impairment of HPA axis regulation is a hallmark of chronic symptomatic psychopathologies whereas immune system may be down regulated or upregulated during an event of stress. Infection, pregnancy and other physiological stressors are characterized by elevated corticosteroid levels as well as augmented inflammatory markers. The cytokine HPA axis interaction has been demonstrated by reports of an elevation of HPA axis activity following injection of pro-inflammatory cytokine IL-6 in monkeys (Reyes, T. M. and Coe, C. L., 1998) as well as humans (Mastorakos, G., Weber, J. S., Magiakou, M. A. *et al*, 1994). Mood blunting has also been reported to be a feature of HPA-axis impairment accompanied by higher concentration of IL-1β in cerebrospinal fluid (Fitzgerald, L., 2011). It has also been postulated that glucocorticoids function as negative feedback control for inflammatory cytokine release as they do for HPA axis itself (Almawi, W. Y., Beyhum, H. N., Rahme, A. A. *et al*, 1996) which may allow us to hypothesize a failure negative feedback control of cytokine cascade concurrent with that of HPA axis. In view of our results, a behavioral modification by modulation of two important determinants of a subject's mood becomes quite logical.

Albeit with a weaker antagonist of P2X7Rs, our results highlight the role of this receptor function in the recovery process from a depressive-like state in mice, supposedly by reversing the inflammatory as well as neuroimmune alterations brought about by stress. The antagonist has been used previously for P2X7R antagonism and has yielded encouraging results in behavioral as well as other experimental paradigms (Jiang, L. H., Mackenzie, A. B., North, R. A. *et al*, 2000;Csolle, C., Ando, R. D., Kittel, A., Goloncser, F., Baranyi, M., Soproni, K., Zelena, D., Haller, J., Nemeth, T., Mocsai, A., and Sperlagh, B., 2012). The recovery process brought about by P2X7 receptor antagonism was independent of any effect on number of new neurons in the dentate gyrus in the mice. A behavioral or to be more specific, antidepressant effect independent of neurogenesis has interesting

implication for future pharmacotherapy of depression. Current regime of first line antidepressant medication (SSRIs) has its limitations in terms of relapse of symptoms after cessation of treatment, resistance as well as side effects such as sexual dysfunction, weight gain and insomnia (Cascade, E., Kalali, A. H., and Kennedy, S. H., 2009). SSRIs induced side effects such as sexual dysfunction, insomnia and weight gain are also causing a great burden for the patients and their physicians. In this regard antagonism of P2X7 receptors presents a potential modus operandi which can be exploited for newer drug development.

7.3. Endocrine-Immune interaction with neurogenesis

Major depression has been consistently characterized by decrease in neurogenesis along with alterations in the regulation of corticosteroid secretion (Zunszain, P. A., Anacker, C., Cattaneo, A. *et al*, 2011). Decrease in neurogenesis has long been the focus of the drug development for major depressive disorder and has undoubtedly been helping patients over last decades. So it is appropriate to relate any new findings to the already established features of depression such as neurogenesis.

Decreased neurogenesis may occur as a result of many factors including a decrease in concentration in monoamines around the synapses, down regulation of serotonin receptors, dysfunction of serotonin transporters or a potential activation of an alternate pathway of amine metabolism (Leonard, B. and Maes, M., 2012). Alteration in neurogenesis may also occur under the influence of altered inflammatory and/or neuroendocrine regulation (Russo, I., Barlati, S., and Bosetti, F., 2011;Kunugi, H., Hori, H., Adachi, N. *et al*, 2010). It is however, difficult to ascertain which of these changes precedes others. Hippocampal lesions and decrease in neurogenesis has been shown to result in neuroendocrine dysregulation (Snyder, J. S., Soumier, A., Brewer, M. *et al*, 2011). Moreover hippocampal CA3 lesion induced spatial memory deficit, attributed to a consequent elevation of adrenocorticotropin and corticosterone, was successfully reverted using metyrapone, a synthesis inhibitor of corticosteroids (Roozendaal, B., Phillips, R. G., Power, A. E. *et al*, 2001). On the other hand, data also exists suggesting that it is actually the alterations in the neuroendocrine system that induce a decrease in neurogenesis (for a

review see (Mirescu, C. and Gould, E., 2006). Inflammation, like its universal character, is protective in nature and is supposed to enhance neurogenesis but when sustained for longer period of time, its character changes and it secretes higher quantities of inflammatory agents which are detrimental to new born neurons (for a review see (Bruno P.Carreira, Maria Inês Morte, Caetana M.Carvalho *et al*, 2012). Decrease in neurogenesis alone or in conjunction with other factors like neuroendocrine or inflammatory alterations influence the behavior of the organism. Role of neuroimmune as well as neuroendocrine pathways needs to be explored further in order to highlight their dynamics and interaction with the process of neurogenesis which might permit us to see beyond a monoamine based therapeutic approach to depressive illness. As discussed earlier, a focus on re-establishing neurogenesis alone has been only a partial solution to this problem *(as discussed in section 1.2 Epidemiology)*.

Dysregulation of diurnal HPA-axis or its negative feedback control (Dallman, M. F., 1993) under stressful conditions causes alterations in the homeostatic environment of the organism (Strohle, A. and Holsboer, F., 2003), including an impact on subject's mood via its interaction with neurogenic and/or neuroinflammatory pathways (Wong, E. Y. and Herbert, J., 2004;Harrison, C., 2011). Indeed any attempt to reverse this cascade of processes would include a possible reversal of all of these factors, individually or together. SSRIs, apart from having pro-neurogenic effects, are also effective at correcting neuroendocrine and neuroinflammatory components of depressive illness (Nikisch, G., 2009;Hashioka, S., 2011). However, it has also been noted that persistent HPA axis alterations may be a predictor of relapse of the symptoms (Zobel, A. W., Nickel, T., Sonntag, A. *et al*, 2001;Zobel, A. W., Yassouridis, A., Frieboes, R. M. *et al*, 1999). It is thus legitimate proposition that a more specific and direct pharmacological targeting of neuroendocrine perturbations, possibly in a group of patients identified with specific risk factors, such as an antagonism of a participating receptor function should be deemed optimal for a complete remission.

Many regulators of HPA axis have been tested for their potential antidepressant effect. An interesting case in this regard is of a repeatedly

suicidal depressed young female who had elevated cortisol level and impairment of dexamethasone suppression. She was treated with steroid suppressors which resulted in complete and sustained remission of the symptoms (Murphy, B. E., 1991). Other agents in this category tested and found to be effective in sub-groups of patients include glucocorticoid receptor antagonists, CRF1 receptor antagonists and vasopressin V1b receptor antagonists (reviewed in (Thomson, F. and Craighead, M., 2008).

Interaction of HPA axis with inflammatory mediators such as IL-1β and other cytokines have been discussed in the previous section. Data regarding P2X7 receptor function and neuroendocrine regulation alteration is yet to be collected in humans as well as in animal models. Upcoming results of our experiments regarding P2X7 receptor expression and their correlation with the already concluded dexamethasone suppression test may well be the first evidence of this interaction. The interesting part of the debate, the one sided interaction between microglial activation and neurogenesis, is discussed as under.

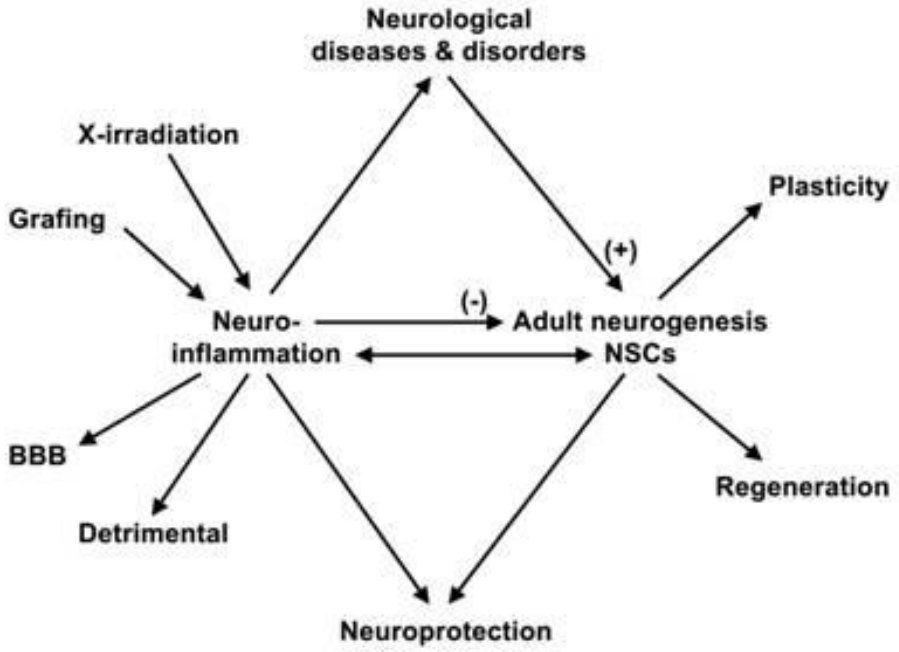

Figure 13: A schematic representation of neuroinflammation and its potential effects on neurogenesis (Taupin, P., 2008).

As shown in the figure 15, neuroinflammation affects adult neurogenesis with all its adversities. Microglia activated by LPS phagocytize viable

neurons, which makes phagocytosis cause and not the consequence of cell death (Neher, J. J., Neniskyte, U., and Brown, G. C., 2012). New born and maturing population of neurons is especially at risk of the deleterious effects of neuroinflammation (Ekdahl, C. T., Claasen, J. H., Bonde, S. *et al*, 2003;Monje, M. L., Toda, H., and Palmer, T. D., 2003). Excessive microglial activity in the hippocampal region, as observed in our first experiment, is an evidence of their direct implication in the associated depressive like behavior. Blocking of microglial activity, as for example with minocycline has also been reported to ameliorate cognition and mood symptoms (Nikodemova, M., Watters, J. J., Jackson, S. J. *et al*, 2007;Hinwood, M., Morandini, J., Day, T. A. *et al*, 2012). Despite many instances of evidence reported for a microglial influence on neurogenesis (Biscaro, B., Lindvall, O., Tesco, G. *et al*, 2012) also reviewed in {Tonchev, 2011 1 /id}, their dynamics in no way fall secondary to the alterations in the ongoing process of neurogenesis which makes it an independent mechanism of behavioral modulation awaiting intervention. Human data regarding evidence of neuroinflammation is still lacking. Only a handful of studies have explored this parameters in depressed subjects mostly in co-morbid conditions. One such study reported microglial activation in depressed schizophrenics who committed suicide {Steiner, 2008 1 /id} (REF PMID 17174336). Newer non-invasive markers of neuroinflammation will help collect data in clinical samples in near future.

Our results propose a fresh approach to find a therapeutic novelty which attempts to limit the deleterious consequences of inflammatory as well as neuroendocrine components of depressive disorder. These results will help integrate endocrine and inflammatory dynamics of brain in the pathophysiology as well as pharmacotherapy of depressive illness. It can be argued that such an approach can help establish a spectrum of pharmacological agents which will primarily target those determinants of a subject's mood which are independent of monoamine/neurogenesis based alterations. These potential targets of intervention may have advantage over their predecessors in terms of undesirable effects, compliance and effectivity. These results will help integrate endocrine and inflammatory dynamics of brain in the pathophysiology of depressive illness.

Limitations of our work are indeed many. Our first experiment lacked a group with antidepressant treatment. Moreover, the evaluation of depressive-like behavior only consisted of coat state score. Behavior tests showing other features of UCMS induced depressive-like syndrome would have had added to the value of these results. We attributed lack of peripheral pro-inflammatory alterations to a methodological difference as we used serum for this evaluation instead of plasma although evidence for this component as a necessary component seems too general and vague. We faced financial difficulty in obtaining a more specific antagonist of P2X7 receptor given the number of animals and duration of their treatment planned for this study. Our results with double-cortin lack an effect of UCMS on this neuronal population. We might expect an effect of BBG on this parameter had there been an effect of stress. We are still in the process of carrying out immunohistochemistry for microglial activation as well as for any differences in P2X7 receptor expression on mice brains which would undoubtedly add to the overall worth and application of these results in the long run.

7.4. Conclusion

Inflammatory component of depression has amassed so much evidence that it is impossible to deny it a role in pharmacotherapy of depressive illness. So it is not very farther when we can anticipate screening of patients for these markers before treatment initiation and availability of flexible pharmacological strategies and recommendations accommodating these alterations. Results of our first and second UCMS experiments, when seen in continuation, strongly suggest that exposure to UCMS induces a depressive like behavior in mice. This depressive like behavior is accompanied by a higher degree of microglial activation in stress responsive regions of mice brain as well as alterations of HPA axis. Peripheral pro-inflammatory activity is spared of any effect of this protocol. Moreover, blocking this process at a receptor level indispensable to the activation of one of the chief player of the inflammatory cascade is an optimal strategy to reverse the whole syndrome of behavioral and neuroendocrine modifications which can potentially present a point of intervention with a better efficacy and safety profile compared to the

existing drugs of choice.

7.5. Perspectives

Our results have opened a potentially new perspective of looking at the pharmacotherapy of major depression. Yet there is a long way to go in order to make a clinical impact with these results. Next most pertinent step following these results may be to relate these findings to the expression of P2X7 receptor, IL-1β as well as microglial activation in the mice brain. Animal models using more specific and effective antagonists of P2X7 receptors, with better penetrability of blood brain barrier as well as transgenic animal may also help explore valuable and more specific information regarding its function. It would also be pertinent to look for evidence and dynamics of neuroinflammation in depressed subjects and relating them to the neuroendocrine alterations. Identifying predictors, risk factors and markers of dominance of this phenomenon in humans will greatly help to screen out patients at risk of developing resistance to conventional monoamine based antidepressant or relapse of symptoms. Another interesting implication of these results would be the recording of HPA-axis dynamics in individuals presenting with SNPs of P2RX7 gene implicated in depressive illness. A suitable pharmacological antagonist of P2X7 receptors capable of reversing stress related consequences and an acceptable safety profile would add a useful weapon to our antidepressant

References

1. World Health Organization. Depression. 2012. 30-8-2012.

2. Aarum, J, Sandberg, K, Haeberlein, SL, Persson, MA Migration and differentiation of neural precursor cells can be directed by microglia. *Proc Natl Acad Sci U S A* 2003; **100:** 15983-88.

3. Abbott, NJ Inflammatory mediators and modulation of blood-brain barrier permeability. *Cell Mol Neurobiol* 2000; **20:** 131-47.

4. Abbracchio, MP, Burnstock, G Purinoceptors: are there families of P2X and P2Y purinoceptors? *Pharmacol Ther* 1994; **64:** 445-75.

5. Abbracchio, MP, Burnstock, G Purinergic signalling: pathophysiological roles. *Jpn J Pharmacol* 1998; **78:** 113-45.

6. Abdulla, YH, Hamadah, K Effect of ADP on PGE1 formation in blood platelets from patients with depression, mania and schizophrenia. *Br J Psychiatry* 1975; **127:** 591-95.

7. Abraham, J, Johnson, RW Central inhibition of interleukin-1beta ameliorates sickness behavior in aged mice. *Brain Behav Immun* 2009; **23:** 396-401.

8. Agnello, D, Villa, P, Ghezzi, P Increased tumor necrosis factor and interleukin-6 production in the central nervous system of interleukin-10-deficient mice. *Brain Res* 2000; **869:** 241-43.

9. Aguglia, E, Ravasio, R, Simonetti, M, Pecchioli, S, Mazzoleni, F Use and treatment modalities for SSRI and SNRI antidepressants in Italy during the period 2003-2009. *Curr Med Res Opin* 2012.

10. Ahmad, A, Rasheed, N, Banu, N, Palit, G Alterations in monoamine levels and oxidative systems in frontal cortex, striatum, and hippocampus of the rat brain during chronic unpredictable stress. *Stress* 2010; **13:** 355-64.

11. Ahrens, B, Linden, M Is there a suicidality syndrome independent of specific major psychiatric disorder? Results of a split half multiple regression analysis. *Acta Psychiatr Scand* 1996; **94:** 79-86.

12. Akhondzadeh, S, Jafari, S, Raisi, F, Nasehi, AA, Ghoreishi, A, Salehi, B, Mohebbi-Rasa, S, Raznahan, M, Kamalipour, A Clinical trial of adjunctive celecoxib treatment in patients with major depression: a double blind and placebo controlled trial. *Depress Anxiety* 2009; **26:** 607-11.

13. Akira, S, Takeda, K, Kaisho, T Toll-like receptors: critical proteins linking innate and acquired immunity. *Nat Immunol* 2001; **2:** 675-

80.

14. Akira, S, Uematsu, S, Takeuchi, O Pathogen recognition and innate immunity. *Cell* 2006; **124:** 783-801.

15. Al-Harbi, KS Treatment-resistant depression: therapeutic trends, challenges, and future directions. *Patient Prefer Adherence* 2012; **6:** 369-88.

16. Alexopoulos, GS, Meyers, BS, Young, RC, Campbell, S, Silbersweig, D, Charlson, M 'Vascular depression' hypothesis. *Arch Gen Psychiatry* 1997a; **54:** 915-22.

17. Alexopoulos, GS, Meyers, BS, Young, RC, Kakuma, T, Silbersweig, D, Charlson, M Clinically defined vascular depression. *Am J Psychiatry* 1997b; **154:** 562-65.

18. Alexopoulos, GS, Morimoto, SS The inflammation hypothesis in geriatric depression. *Int J Geriatr Psychiatry* 2011.

19. Amrein, R, Martin, JR, Cameron, AM Moclobemide in patients with dementia and depression. *Adv Neurol* 1999; **80:** 509-19.

20. Anderson, IM Meta-analytical studies on new antidepressants. *Br Med Bull* 2001; **57:** 161-78.

21. Anderson, IM, Tomenson, BM The efficacy of selective serotonin re-uptake inhibitors in depression: a meta-analysis of studies against tricyclic antidepressants. *J Psychopharmacol* 1994; **8:** 238-49.

22. Anderson, IM, Tomenson, BM Treatment discontinuation with selective serotonin reuptake inhibitors compared with tricyclic antidepressants: a meta-analysis. *BMJ* 1995; **310:** 1433-38.

23. Anderson, RJ, Freedland, KE, Clouse, RE, Lustman, PJ The prevalence of comorbid depression in adults with diabetes: a meta-analysis. *Diabetes Care* 2001a; **24:** 1069-78.

24. Anderson, RJ, Freedland, KE, Clouse, RE, Lustman, PJ The prevalence of comorbid depression in adults with diabetes: a meta-analysis. *Diabetes Care* 2001b; **24:** 1069-78.

25. Angold, A, Costello, EJ, Worthman, CM Puberty and depression: the roles of age, pubertal status and pubertal timing. *Psychol Med* 1998; **28:** 51-61.

26. Angst, F, Stassen, HH, Clayton, PJ, Angst, J Mortality of patients with mood disorders: follow-up over 34-38 years. *J Affect Disord* 2002; **68:** 167-81.

27. Anisman, H, Ravindran, AV, Griffiths, J, Merali, Z Endocrine and cytokine correlates of major depression and dysthymia with typical or atypical features. *Mol Psychiatry* 1999; **4:** 182-88.

28. Arend, WP, Gabay, C Physiologic role of interleukin-1 receptor antagonist. *Arthritis Res* 2000; **2:** 245-48.

29. Armstrong, JN, Brust, TB, Lewis, RG, MacVicar, BA Activation of presynaptic P2X7-like receptors depresses mossy fiber-CA3 synaptic transmission through p38 mitogen-activated protein kinase. *J Neurosci* 2002; **22:** 5938-45.

30. Arshad, M, Arham, AZ, Arif, M, Bano, M, Bashir, A, Bokutz, M, Choudhary, MM, Naqvi, H, Khan, MM Awareness and perceptions of electroconvulsive therapy among psychiatric patients: a cross-sectional survey from teaching hospitals in Karachi, Pakistan. *BMC Psychiatry* 2007; **7:** 27.

31. Arulkumaran, N, Unwin, RJ, Tam, FW A potential therapeutic role for P2X7 receptor (P2X7R) antagonists in the treatment of inflammatory diseases. *Expert Opin Investig Drugs* 2011; **20:** 897-915.

32. ASATOOR, AM, LEVI, AJ, MILNE, MD TRANYLCYPROMINE AND CHEESE. *Lancet* 1963; **2:** 733-34.

33. Asberg, M, Traskman, L, Thoren, P 5-HIAA in the cerebrospinal fluid. A biochemical suicide predictor? *Arch Gen Psychiatry* 1976; **33:** 1193-97.

34. Atmaca, M, Korkmaz, S, Topuz, M, Mermi, O Mirtazapine augmentation for selective serotonin reuptake inhibitor-induced sexual dysfunction: a retropective investigation. *Psychiatry Investig* 2011; **8:** 55-57.

35. Autry, AE, Adachi, M, Nosyreva, E, Na, ES, Los, MF, Cheng, PF, Kavalali, ET, Monteggia, LM NMDA receptor blockade at rest triggers rapid behavioural antidepressant responses. *Nature* 2011; **475:** 91-95.

36. AXELROD, J, HERTTING, G, POTTER, L Effect of drugs on the uptake and release of 3H-norepinephrine in the rat heart. *Nature* 1962; **194:** 297.

37. Bakish, D New standard of depression treatment: remission and full recovery. *J Clin Psychiatry* 2001; **62 Suppl 26:** 5-9.

38. Ballenger, JC, Davidson, JR, Lecrubier, Y, Nutt, DJ, Kirmayer, LJ, Lepine, JP, Lin, KM, Tajima, O, Ono, Y Consensus statement on transcultural issues in depression and anxiety from the International Consensus Group on Depression and Anxiety. *J Clin Psychiatry* 2001; **62 Suppl 13:** 47-55.

39. Banks, WA Blood-brain barrier transport of cytokines: a mechanism for neuropathology. *Curr Pharm Des* 2005; **11:** 973-84.

40. Banks, WA, Kastin, AJ, Broadwell, RD Passage of cytokines across

the blood-brain barrier. *Neuroimmunomodulation* 1995; **2:** 241-48.

41. Barden, N, Harvey, M, Gagne, B, Shink, E, Tremblay, M, Raymond, C, Labbe, M, Villeneuve, A, Rochette, D, Bordeleau, L, Stadler, H, Holsboer, F, Muller-Myhsok, B Analysis of single nucleotide polymorphisms in genes in the chromosome 12Q24.31 region points to P2RX7 as a susceptibility gene to bipolar affective disorder. *Am J Med Genet B Neuropsychiatr Genet* 2006a; **141B:** 374-82.

42. Barden, N, Harvey, M, Gagne, B, Shink, E, Tremblay, M, Raymond, C, Labbe, M, Villeneuve, A, Rochette, D, Bordeleau, L, Stadler, H, Holsboer, F, Muller-Myhsok, B Analysis of single nucleotide polymorphisms in genes in the chromosome 12Q24.31 region points to P2RX7 as a susceptibility gene to bipolar affective disorder. *Am J Med Genet B Neuropsychiatr Genet* 2006b; **141B:** 374-82.

43. Barrientos, RM, Sprunger, DB, Campeau, S, Higgins, EA, Watkins, LR, Rudy, JW, Maier, SF Brain-derived neurotrophic factor mRNA downregulation produced by social isolation is blocked by intrahippocampal interleukin-1 receptor antagonist. *Neuroscience* 2003; **121:** 847-53.

44. Basso, AM, Bratcher, NA, Harris, RR, Jarvis, MF, Decker, MW, Rueter, LE Behavioral profile of P2X7 receptor knockout mice in animal models of depression and anxiety: relevance for neuropsychiatric disorders. *Behav Brain Res* 2009; **198:** 83-90.

45. Battista, D, Ferrari, CC, Gage, FH, Pitossi, FJ Neurogenic niche modulation by activated microglia: transforming growth factor beta increases neurogenesis in the adult dentate gyrus. *Eur J Neurosci* 2006; **23:** 83-93.

46. Beall, EB, Malone, DA, Dale, RM, Muzina, DJ, Koenig, KA, Bhattacharrya, PK, Jones, SE, Phillips, MD, Lowe, MJ Effects of Electroconvulsive Therapy on Brain Functional Activation and Connectivity in Depression. *J ECT* 2012.

47. Beauregard, M, Paquette, V, Levesque, J Dysfunction in the neural circuitry of emotional self-regulation in major depressive disorder. *Neuroreport* 2006; **17:** 843-46.

48. Becker, G, Berg, D, Lesch, KP, Becker, T Basal limbic system alteration in major depression: a hypothesis supported by transcranial sonography and MRI findings. *Int J Neuropsychopharmacol* 2001; **4:** 21-31.

49. Belarbi, K, Arellano, C, Ferguson, R, Jopson, T, Rosi, S Chronic neuroinflammation impacts the recruitment of adult-born neurons into behaviorally relevant hippocampal networks. *Brain Behav Immun* 2012; **26:** 18-23.

50. Belzung, C, Lemoine, M Criteria of validity for animal models of

psychiatric disorders: focus on anxiety disorders and depression. *Biol Mood Anxiety Disord* 2011a; **1:** 9.

51. Belzung, C, Lemoine, M Criteria of validity for animal models of psychiatric disorders: focus on anxiety disorders and depression. *Biol Mood Anxiety Disord* 2011b; **1:** 9.

52. Ben Menachem-Zidon, O, Goshen, I, Kreisel, T, Ben, MY, Reinhartz, E, Ben, HT, Yirmiya, R Intrahippocampal transplantation of transgenic neural precursor cells overexpressing interleukin-1 receptor antagonist blocks chronic isolation-induced impairment in memory and neurogenesis. *Neuropsychopharmacology* 2008; **33:** 2251-62.

53. Bennett, MR Synaptic P2X7 receptor regenerative-loop hypothesis for depression. *Aust N Z J Psychiatry* 2007; **41:** 563-71.

54. Benveniste, EN Role of macrophages/microglia in multiple sclerosis and experimental allergic encephalomyelitis. *J Mol Med (Berl)* 1997; **75:** 165-73.

55. Berg, A, Palomaki, H, Lonnqvist, J, Lehtihalmes, M, Kaste, M Depression among caregivers of stroke survivors. *Stroke* 2005; **36:** 639-43.

56. Berken, GH, Weinstein, DO, Stern, WC Weight gain. A side-effect of tricyclic antidepressants. *J Affect Disord* 1984; **7:** 133-38.

57. Berkenbosch, F, van, OJ, del, RA, Tilders, F, Besedovsky, H Corticotropin-releasing factor-producing neurons in the rat activated by interleukin-1. *Science* 1987; **238:** 524-26.

58. Berman, RM, Cappiello, A, Anand, A, Oren, DA, Heninger, GR, Charney, DS, Krystal, JH Antidepressant effects of ketamine in depressed patients. *Biol Psychiatry* 2000; **47:** 351-54.

59. Bermingham, R, Carballedo, A, Lisiecka, D, Fagan, A, Morris, D, Fahey, C, Donohoe, G, Meaney, J, Gill, M, Frodl, T Effect of Genetic Variant in BICC1 on Functional and Structural Brain Changes in Depression. *Neuropsychopharmacology* 2012.

60. Bernton, EW, Beach, JE, Holaday, JW, Smallridge, RC, Fein, HG Release of multiple hormones by a direct action of interleukin-1 on pituitary cells. *Science* 1987; **238:** 519-21.

61. Bessis, A, Bechade, C, Bernard, D, Roumier, A Microglial control of neuronal death and synaptic properties. *Glia* 2007; **55:** 233-38.

62. Bhagya, V, Srikumar, BN, Raju, TR, Rao, BS Chronic escitalopram treatment restores spatial learning, monoamine levels, and hippocampal long-term potentiation in an animal model of depression. *Psychopharmacology (Berl)* 2011; **214:** 477-94.

63. Bhargava, Y, Rettinger, J, Mourot, A Allosteric nature of P2X receptor activation probed by photoaffinity labeling. *Br J Pharmacol*

2012.

64. Bhatia, SK, Bhatia, SC Childhood and adolescent depression. *Am Fam Physician* 2007; **75:** 73-80.

65. Bhugra, D, Mastrogianni, A Globalisation and mental disorders. Overview with relation to depression. *Br J Psychiatry* 2004; **184:** 10-20.

66. Bhui, K, Bhugra, D Transcultural psychiatry: some social and epidemiological research issues. *Int J Soc Psychiatry* 2001; **47:** 1-9.

67. Binder, E, Malki, K, Paya-Cano, JL, Fernandes, C, Aitchison, KJ, Mathe, AA, Sluyter, F, Schalkwyk, LC Antidepressants and the resilience to early-life stress in inbred mouse strains. *Pharmacogenet Genomics* 2011; **21:** 779-89.

68. Birmaher, B, Brent, D, Bernet, W, Bukstein, O, Walter, H, Benson, RS, Chrisman, A, Farchione, T, Greenhill, L, Hamilton, J, Keable, H, Kinlan, J, Schoettle, U, Stock, S, Ptakowski, KK, Medicus, J Practice parameter for the assessment and treatment of children and adolescents with depressive disorders. *J Am Acad Child Adolesc Psychiatry* 2007; **46:** 1503-26.

69. Bjerkeset, O, Romundstad, P, Gunnell, D Gender differences in the association of mixed anxiety and depression with suicide. *Br J Psychiatry* 2008; **192:** 474-75.

70. Bjorntorp, P Behavior and metabolic disease. *Int J Behav Med* 1996; **3:** 285-302.

71. Black, PH, Garbutt, LD Stress, inflammation and cardiovascular disease. *J Psychosom Res* 2002; **52:** 1-23.

72. Blazer, DG, Hybels, CF Origins of depression in later life. *Psychol Med* 2005; **35:** 1241-52.

73. Block, ML, Zecca, L, Hong, JS Microglia-mediated neurotoxicity: uncovering the molecular mechanisms. *Nat Rev Neurosci* 2007; **8:** 57-69.

74. Bluthe, RM, Castanon, N, Pousset, F, Bristow, A, Ball, C, Lestage, J, Michaud, B, Kelley, KW, Dantzer, R Central injection of IL-10 antagonizes the behavioural effects of lipopolysaccharide in rats. *Psychoneuroendocrinology* 1999; **24:** 301-11.

75. Bluthe, RM, Michaud, B, Kelley, KW, Dantzer, R Vagotomy attenuates behavioural effects of interleukin-1 injected peripherally but not centrally. *Neuroreport* 1996; **7:** 1485-88.

76. Borkowska, P, Kucia, K, Rzezniczek, S, Paul-Samojedny, M, Kowalczyk, M, Owczarek, A, Suchanek, R, Medrala, T, Kowalski, J Interleukin-1beta promoter (-31T/C and -511C/T) polymorphisms

in major recurrent depression. *J Mol Neurosci* 2011; **44:** 12-16.

77. Boucher, AA, Arnold, JC, Hunt, GE, Spiro, A, Spencer, J, Brown, C, McGregor, IS, Bennett, MR, Kassiou, M Resilience and reduced c-Fos expression in P2X7 receptor knockout mice exposed to repeated forced swim test. *Neuroscience* 2011; **189:** 170-177.

78. Brandle, U, Kohler, K, Wheeler-Schilling, TH Expression of the P2X7-receptor subunit in neurons of the rat retina. *Brain Res Mol Brain Res* 1998; **62:** 106-9.

79. BRODIE, BB, BICKEL, MH, SULSER, F Desmethylimipramine, a new type of antidepressant drug. *Med Exp Int J Exp Med* 1961; **5:** 454-58.

80. Brody, AL, Barsom, MW, Bota, RG, Saxena, S Prefrontal-subcortical and limbic circuit mediation of major depressive disorder. *Semin Clin Neuropsychiatry* 2001; **6:** 102-12.

81. Brown, ES, Varghese, FP, McEwen, BS Association of depression with medical illness: does cortisol play a role? *Biol Psychiatry* 2004; **55:** 1-9.

82. Brown, GC, Neher, JJ Eaten alive! Cell death by primary phagocytosis: 'phagoptosis'. *Trends Biochem Sci* 2012.

83. Brunello, N, Alboni, S, Capone, G, Benatti, C, Blom, JM, Tascedda, F, Kriwin, P, Mendlewicz, J Acetylsalicylic acid accelerates the antidepressant effect of fluoxetine in the chronic escape deficit model of depression. *Int Clin Psychopharmacol* 2006; **21:** 219-25.

84. Brunner, HG, Nelen, M, Breakefield, XO, Ropers, HH, van Oost, BA Abnormal behavior associated with a point mutation in the structural gene for monoamine oxidase A. *Science* 1993; **262:** 578-80.

85. Brunner, HG, Nelen, MR, van, ZP, Abeling, NG, van Gennip, AH, Wolters, EC, Kuiper, MA, Ropers, HH, van Oost, BA X-linked borderline mental retardation with prominent behavioral disturbance: phenotype, genetic localization, and evidence for disturbed monoamine metabolism. *Am J Hum Genet* 1993; **52:** 1032-39.

86. Brunoni, AR, Lopes, M, Fregni, F A systematic review and meta-analysis of clinical studies on major depression and BDNF levels: implications for the role of neuroplasticity in depression. *Int J Neuropsychopharmacol* 2008; **11:** 1169-80.

87. Buchheim, A, Viviani, R, Kessler, H, Kachele, H, Cierpka, M, Roth, G, George, C, Kernberg, OF, Bruns, G, Taubner, S Changes in prefrontal-limbic function in major depression after 15 months of long-term psychotherapy. *PLoS One* 2012; **7:** e33745.

88. Budihardjo, I, Oliver, H, Lutter, M, Luo, X, Wang, X Biochemical

pathways of caspase activation during apoptosis. *Annu Rev Cell Dev Biol* 1999; **15:** 269-90.

89. Buell, G, Chessell, IP, Michel, AD, Collo, G, Salazzo, M, Herren, S, Gretener, D, Grahames, C, Kaur, R, Kosco-Vilbois, MH, Humphrey, PP Blockade of human P2X7 receptor function with a monoclonal antibody. *Blood* 1998; **92:** 3521-28.

90. Buell, GN, Talabot, F, Gos, A, Lorenz, J, Lai, E, Morris, MA, Antonarakis, SE Gene structure and chromosomal localization of the human P2X7 receptor. *Receptors Channels* 1998; **5:** 347-54.

91. Burnstock, G, Kennedy, C Is there a basis for distinguishing two types of P2-purinoceptor? *Gen Pharmacol* 1985; **16:** 433-40.

92. Buttini, M, Boddeke, H Peripheral lipopolysaccharide stimulation induces interleukin-1 beta messenger RNA in rat brain microglial cells. *Neuroscience* 1995; **65:** 523-30.

93. Bymaster, FP, Dreshfield-Ahmad, LJ, Threlkeld, PG, Shaw, JL, Thompson, L, Nelson, DL, Hemrick-Luecke, SK, Wong, DT Comparative affinity of duloxetine and venlafaxine for serotonin and norepinephrine transporters in vitro and in vivo, human serotonin receptor subtypes, and other neuronal receptors. *Neuropsychopharmacology* 2001; **25:** 871-80.

94. Byrne, GI, Lehmann, LK, Kirschbaum, JG, Borden, EC, Lee, CM, Brown, RR Induction of tryptophan degradation in vitro and in vivo: a gamma-interferon-stimulated activity. *J Interferon Res* 1986; **6:** 389-96.

95. Cacci, E, Claasen, JH, Kokaia, Z Microglia-derived tumor necrosis factor-alpha exaggerates death of newborn hippocampal progenitor cells in vitro. *J Neurosci Res* 2005; **80:** 789-97.

96. Calabrese, F, Molteni, R, Racagni, G, Riva, MA Neuronal plasticity: a link between stress and mood disorders. *Psychoneuroendocrinology* 2009; **34 Suppl 1:** S208-S216.

97. Cameron, HA, Gould, E Adult neurogenesis is regulated by adrenal steroids in the dentate gyrus. *Neuroscience* 1994; **61:** 203-9.

98. Camus, V, Kraehenbuhl, H, Preisig, M, Bula, CJ, Waeber, G Geriatric depression and vascular diseases: what are the links? *J Affect Disord* 2004a; **81:** 1-16.

99. Camus, V, Kraehenbuhl, H, Preisig, M, Bula, CJ, Waeber, G Geriatric depression and vascular diseases: what are the links? *J Affect Disord* 2004b; **81:** 1-16.

100. Carlsson, A, Lindqvist, M, Magnusson, T 3,4-Dihydroxyphenylalanine and 5-hydroxytryptophan as reserpine antagonists. *Nature* 1957; **180:** 1200.

101. Carroll, Curtis, GC, Mendels, J Neuroendocrine regulation in depression. I. Limbic system-adrenocortical dysfunction. *Arch Gen Psychiatry* 1976; **33:** 1039-44.

102. Carroll, BJ Dexamethasone suppression test for depression. *Adv Biochem Psychopharmacol* 1984; **39:** 179-88.

103. Carroll, BJ, Martin, FI, Davies, B Pituitary-adrenal function in depression. *Lancet* 1968; **1:** 1373-74.

104. Casacalenda, N, Perry, JC, Looper, K Remission in major depressive disorder: a comparison of pharmacotherapy, psychotherapy, and control conditions. *Am J Psychiatry* 2002; **159:** 1354-60.

105. Cases, O, Seif, I, Grimsby, J, Gaspar, P, Chen, K, Pournin, S, Muller, U, Aguet, M, Babinet, C, Shih, JC, . Aggressive behavior and altered amounts of brain serotonin and norepinephrine in mice lacking MAOA. *Science* 1995; **268:** 1763-66.

106. Cases, O, Vitalis, T, Seif, I, De, ME, Sotelo, C, Gaspar, P Lack of barrels in the somatosensory cortex of monoamine oxidase A-deficient mice: role of a serotonin excess during the critical period. *Neuron* 1996; **16:** 297-307.

107. Caspi, A, Sugden, K, Moffitt, TE, Taylor, A, Craig, IW, Harrington, H, McClay, J, Mill, J, Martin, J, Braithwaite, A, Poulton, R Influence of life stress on depression: moderation by a polymorphism in the 5-HTT gene. *Science* 2003; **301:** 386-89.

108. Castren, E Is mood chemistry? *Nat Rev Neurosci* 2005a; **6:** 241-46.

109. Castren, E Is mood chemistry? *Nat Rev Neurosci* 2005b; **6:** 241-46.

110. Chadman, KK, Yang, M, Crawley, JN Criteria for validating mouse models of psychiatric diseases. *Am J Med Genet B Neuropsychiatr Genet* 2009; **150B:** 1-11.

111. Chakfe, Y, Seguin, R, Antel, JP, Morissette, C, Malo, D, Henderson, D, Seguela, P ADP and AMP induce interleukin-1beta release from microglial cells through activation of ATP-primed P2X7 receptor channels. *J Neurosci* 2002; **22:** 3061-69.

112. Chavant, F, Deguil, J, Pain, S, Ingrand, I, Milin, S, Fauconneau, B, Perault-Pochat, MC, Lafay-Chebassier, C Imipramine, in part through tumor necrosis factor alpha inhibition, prevents cognitive decline and beta-amyloid accumulation in a mouse model of Alzheimer's disease. *J Pharmacol Exp Ther* 2010; **332:** 505-14.

113. Chen, G, Wu, Y, Wang, T, Liang, J, Lin, W, Li, L, Wen, J, Lin, L, Huang, H Association Between Serum Endogenous Secretory Receptor for Advanced Glycation End Products and Risk of Type 2 Diabetes Mellitus with Combined Depression in the Chinese Population. *Diabetes Technol Ther* 2012.

114. Chen, H, Yiu, KH, Tse, HF Relationships between vascular dysfunction, circulating endothelial progenitor cells, and psychological status in healthy subjects. *Depress Anxiety* 2011; **28:** 719-27.

115. Cheng, AT Mental illness and suicide. A case-control study in east Taiwan. *Arch Gen Psychiatry* 1995; **52:** 594-603.

116. Chioqueta A.P., Stiles T.C. Personality traits and the development of depression, hopelessness and suicide ideation. *Personality and Individual Differences* 2005; **38:** 1283-91.

117. Chourbaji, S, Vogt, MA, Gass, P Mice that under- or overexpress glucocorticoid receptors as models for depression or posttraumatic stress disorder. *Prog Brain Res* 2008; **167:** 65-77.

118. Chrousos, GP Stressors, stress, and neuroendocrine integration of the adaptive response. The 1997 Hans Selye Memorial Lecture. *Ann N Y Acad Sci* 1998; **851:** 311-35.

119. Cockcroft, S, Gomperts, BD ATP induces nucleotide permeability in rat mast cells. *Nature* 1979; **279:** 541-42.

120. Cohen, S, Janicki-Deverts, D, Miller, GE Psychological stress and disease. *JAMA* 2007; **298:** 1685-87.

121. Collo, G, Neidhart, S, Kawashima, E, Kosco-Vilbois, M, North, RA, Buell, G Tissue distribution of the P2X7 receptor. *Neuropharmacology* 1997a; **36:** 1277-83.

122. Collo, G, Neidhart, S, Kawashima, E, Kosco-Vilbois, M, North, RA, Buell, G Tissue distribution of the P2X7 receptor. *Neuropharmacology* 1997b; **36:** 1277-83.

123. Combrinck, MI, Perry, VH, Cunningham, C Peripheral infection evokes exaggerated sickness behaviour in pre-clinical murine prion disease. *Neuroscience* 2002; **112:** 7-11.

124. Connor, TJ, Starr, N, O'Sullivan, JB, Harkin, A Induction of indolamine 2,3-dioxygenase and kynurenine 3-monooxygenase in rat brain following a systemic inflammatory challenge: a role for IFN-gamma? *Neurosci Lett* 2008; **441:** 29-34.

125. Conwell, Y, Duberstein, PR, Cox, C, Herrmann, JH, Forbes, NT, Caine, ED Relationships of age and axis I diagnoses in victims of completed suicide: a psychological autopsy study. *Am J Psychiatry* 1996; **153:** 1001-8.

126. Coppen, A The biochemistry of affective disorders. *Br J Psychiatry* 1967; **113:** 1237-64.

127. Coppen, A Lithium in unipolar depression and the prevention of suicide. *J Clin Psychiatry* 2000; **61 Suppl 9:** 52-56.

128. Corwin, EJ, Johnston, N, Pugh, L Symptoms of postpartum depression associated with elevated levels of interleukin-1 beta during the first month postpartum. *Biol Res Nurs* 2008; **10:** 128-33.

129. Cousins, RJ, Leinart, AS Tissue-specific regulation of zinc metabolism and metallothionein genes by interleukin 1. *FASEB J* 1988; **2:** 2884-90.

130. Crane, JW, Buller, KM, Day, TA Evidence that the bed nucleus of the stria terminalis contributes to the modulation of hypophysiotropic corticotropin-releasing factor cell responses to systemic interleukin-1beta. *J Comp Neurol* 2003; **467:** 232-42.

131. Csolle, C, Ando, RD, Kittel, A, Goloncser, F, Baranyi, M, Soproni, K, Zelena, D, Haller, J, Nemeth, T, Mocsai, A, Sperlagh, B The absence of P2X7 receptors (P2rx7) on non-haematopoietic cells leads to selective alteration in mood-related behaviour with dysregulated gene expression and stress reactivity in mice. *Int J Neuropsychopharmacol* 2012; 1-21.

132. Csolle, C, Sperlagh, B Peripheral origin of IL-1beta production in the rodent hippocampus under in vivo systemic bacterial lipopolysaccharide (LPS) challenge and its regulation by P2X(7) receptors. *J Neuroimmunol* 2010; **219:** 38-46.

133. Cua, DJ, Groux, H, Hinton, DR, Stohlman, SA, Coffman, RL Transgenic interleukin 10 prevents induction of experimental autoimmune encephalomyelitis. *J Exp Med* 1999; **189:** 1005-10.

134. Cuijpers, P, Dekker, J, Hollon, SD, Andersson, G Adding psychotherapy to pharmacotherapy in the treatment of depressive disorders in adults: a meta-analysis. *J Clin Psychiatry* 2009; **70:** 1219-29.

135. Cullen, K, Klimes-Dougan, B, Kumra, S Pediatric depression: issues and treatment recommendations. *Minn Med* 2009; **92:** 45-48.

136. Culpepper, L Why do you need to move beyond first-line therapy for major depression? *J Clin Psychiatry* 2010; **71 Suppl 1:** 4-9.

137. d'Audiffret, AC Depressive behavior and vascular dysfunction: a link between clinical depression and vascular disease? 2010.

138. d'Audiffret, AC, Frisbee, SJ, Stapleton, PA, Goodwill, AG, Isingrini, E, Frisbee, JC Depressive behavior and vascular dysfunction: a link between clinical depression and vascular disease? *J Appl Physiol* 2010; **108:** 1041-51.

139. Dani, JW, Chernjavsky, A, Smith, SJ Neuronal activity triggers calcium waves in hippocampal astrocyte networks. *Neuron* 1992; **8:** 429-40.

140. Dantzer, R Cytokine-induced sickness behavior: where do we

stand? *Brain Behav Immun* 2001; **15:** 7-24.

141. Dantzer, R, O'Connor, JC, Freund, GG, Johnson, RW, Kelley, KW From inflammation to sickness and depression: when the immune system subjugates the brain. *Nat Rev Neurosci* 2008; **9:** 46-56.

142. Datto, CJ, Oslin, DW, Streim, JE, Scheinthal, SM, DiFilippo, S, Katz, IR Pharmacologic treatment of depression in nursing home residents: a mental health services perspective. *J Geriatr Psychiatry Neurol* 2002; **15:** 141-46.

143. Davalos, D, Grutzendler, J, Yang, G, Kim, JV, Zuo, Y, Jung, S, Littman, DR, Dustin, ML, Gan, WB ATP mediates rapid microglial response to local brain injury in vivo. *Nat Neurosci* 2005; **8:** 752-58.

144. Davis, JM, Wang, Z, Janicak, PG A quantitative analysis of clinical drug trials for the treatment of affective disorders. *Psychopharmacol Bull* 1993; **29:** 175-81.

145. de Burgos-Lunar, C, Gomez-Campelo, P, Cardenas-Valladolid, J, Fuentes-Rodriguez, CY, Granados-Menendez, MI, Lopez-Lopez, F, Salinero-Fort, MA Effect of depression on mortality and cardiovascular morbidity in type 2 diabetes mellitus after 3 years follow up. The DIADEMA study protocol. *BMC Psychiatry* 2012; **12:** 95.

146. de Kloet, ER, Sibug, RM, Helmerhorst, FM, Schmidt, MV Stress, genes and the mechanism of programming the brain for later life. *Neurosci Biobehav Rev* 2005; **29:** 271-81.

147. de Vries, HE, Kuiper, J, de Boer, AG, Van Berkel, TJ, Breimer, DD The blood-brain barrier in neuroinflammatory diseases. *Pharmacol Rev* 1997; **49:** 143-55.

148. De, LD Why are we not getting any closer to preventing suicide? *Br J Psychiatry* 2002; **181:** 372-74.

149. Deak, T, Bordner, KA, McElderry, NK, Barnum, CJ, Blandino, P, Jr., Deak, MM, Tammariello, SP Stress-induced increases in hypothalamic IL-1: a systematic analysis of multiple stressor paradigms. *Brain Res Bull* 2005; **64:** 541-56.

150. Delgado, PL Monoamine depletion studies: implications for antidepressant discontinuation syndrome. *J Clin Psychiatry* 2006; **67 Suppl 4:** 22-26.

151. Denlinger, LC, Fisette, PL, Sommer, JA, Watters, JJ, Prabhu, U, Dubyak, GR, Proctor, RA, Bertics, PJ Cutting edge: the nucleotide receptor P2X7 contains multiple protein- and lipid-interaction motifs including a potential binding site for bacterial lipopolysaccharide. *J Immunol* 2001; **167:** 1871-76.

152. Dentino, AN, Pieper, CF, Rao, MK, Currie, MS, Harris, T, Blazer, DG, Cohen, HJ Association of interleukin-6 and other biologic variables

with depression in older people living in the community. *J Am Geriatr Soc* 1999; **47:** 6-11.

153. Derijk, RH Single nucleotide polymorphisms related to HPA axis reactivity. *Neuroimmunomodulation* 2009; **16:** 340-352.

154. Desmidt, T, Hachemi, ME, Remenieras, JP, Lecomte, P, Ferreira-Maldent, N, Patat, F, Camus, V Ultrasound Brain Tissue Pulsatility is decreased in middle aged and elderly type 2 diabetic patients with depression. *Psychiatry Res* 2011; **193:** 63-64.

155. Dettling, AC, Gunnar, MR, Donzella, B Cortisol levels of young children in full-day childcare centers: relations with age and temperament. *Psychoneuroendocrinology* 1999; **24:** 519-36.

156. Deuchars, SA, Atkinson, L, Brooke, RE, Musa, H, Milligan, CJ, Batten, TF, Buckley, NJ, Parson, SH, Deuchars, J Neuronal P2X7 receptors are targeted to presynaptic terminals in the central and peripheral nervous systems. *J Neurosci* 2001; **21:** 7143-52.

157. DEVERTEUIL, RL, Lehmann, HE Therapeutic trial of iproniazid (marsilid) in depressed and apathetic patients. *Can Med Assoc J* 1958; **78:** 131-33.

158. Di, VF The P2Z purinoceptor: an intriguing role in immunity, inflammation and cell death. *Immunol Today* 1995; **16:** 524-28.

159. Di, VF, Chiozzi, P, Ferrari, D, Falzoni, S, Sanz, JM, Morelli, A, Torboli, M, Bolognesi, G, Baricordi, OR Nucleotide receptors: an emerging family of regulatory molecules in blood cells. *Blood* 2001; **97:** 587-600.

160. Dinarello, CA Biologic basis for interleukin-1 in disease. *Blood* 1996a; **87:** 2095-147.

161. Dinarello, CA Biologic basis for interleukin-1 in disease. *Blood* 1996b; **87:** 2095-147.

162. Dinarello, CA The role of the interleukin-1-receptor antagonist in blocking inflammation mediated by interleukin-1. *N Engl J Med* 2000; **343:** 732-34.

163. Dinarello, CA The IL-1 family and inflammatory diseases. *Clin Exp Rheumatol* 2002; **20:** S1-13.

164. do Amaral, JF, Silva, MI, de Aquino Neto, MR, Moura, BA, de Carvalho, AM, Vasconcelos, PF, Barbosa Filho, JM, Gutierrez, SJ, Vasconcelos, SM, Macedo, DS, de Sousa, FC Antidepressant-like effect of bis-eugenol in the mice forced swimming test: evidence for the involvement of the monoaminergic system. *Fundam Clin Pharmacol* 2012.

165. Do, DP, Dowd, JB, Ranjit, N, House, JS, Kaplan, GA Hopelessness, depression, and early markers of endothelial dysfunction in U.S.

adults. *Psychosom Med* 2010; **72:** 613-19.

166. Dofferhoff, AS, Vellenga, E, Limburg, PC, van, ZA, Mulder, PO, Weits, J Tumour necrosis factor (cachectin) and other cytokines in septic shock: a review of the literature. *Neth J Med* 1991; **39:** 45-62.

167. Dowlati, Y, Herrmann, N, Swardfager, W, Liu, H, Sham, L, Reim, EK, Lanctot, KL A meta-analysis of cytokines in major depression. *Biol Psychiatry* 2010; **67:** 446-57.

168. Downes, CE, Crack, PJ Neural injury following stroke: are Toll-like receptors the link between the immune system and the CNS? *Br J Pharmacol* 2010; **160:** 1872-88.

169. Duan, S, Anderson, CM, Keung, EC, Chen, Y, Chen, Y, Swanson, RA P2X7 receptor-mediated release of excitatory amino acids from astrocytes. *J Neurosci* 2003; **23:** 1320-1328.

170. Duch, DS, Woolf, JH, Nichol, CA, Davidson, JR, Garbutt, JC Urinary excretion of biopterin and neopterin in psychiatric disorders. *Psychiatry Res* 1984; **11:** 83-89.

171. Dunn, AJ, Wang, J, Ando, T Effects of cytokines on cerebral neurotransmission. Comparison with the effects of stress. *Adv Exp Med Biol* 1999; **461:** 117-27.

172. Edwards, E, Kitt, C, Oliver, E, Finkelstein, J, Wagster, M, McDonald, WM Depression and Parkinson's disease: a new look at an old problem. *Depress Anxiety* 2002; **16:** 39-48.

173. Ekdahl, CT, Claasen, JH, Bonde, S, Kokaia, Z, Lindvall, O Inflammation is detrimental for neurogenesis in adult brain. *Proc Natl Acad Sci U S A* 2003a; **100:** 13632-37.

174. Ekdahl, CT, Claasen, JH, Bonde, S, Kokaia, Z, Lindvall, O Inflammation is detrimental for neurogenesis in adult brain. *Proc Natl Acad Sci U S A* 2003b; **100:** 13632-37.

175. Ekdahl, CT, Claasen, JH, Bonde, S, Kokaia, Z, Lindvall, O Inflammation is detrimental for neurogenesis in adult brain. *Proc Natl Acad Sci U S A* 2003c; **100:** 13632-37.

176. El-Alfy, AT, Abourashed, EA, Matsumoto, RR Nature against depression. *Curr Med Chem* 2012; **19:** 2229-41.

177. Elderkin-Thompson, V, Irwin, MR, Hellemann, G, Kumar, A Interleukin-6 and Memory Functions of Encoding and Recall in Healthy and Depressed Elderly Adults. *Am J Geriatr Psychiatry* 2012.

178. Elovainio, M, Hurme, M, Jokela, M, Pulkki-Raback, L, Kivimaki, M, Hintsanen, M, Hintsa, T, Lehtimaki, T, Viikari, J, Raitakari, OT, Keltikangas-Jarvinen, L Indoleamine 2,3-Dioxygenase Activation and Depressive Symptoms: Results From the Young Finns Study.

Psychosom Med 2012.

179. Endemann, DH, Schiffrin, EL Endothelial dysfunction. *J Am Soc Nephrol* 2004; **15:** 1983-92.

180. Epstein, DH, Preston, KL, Stewart, J, Shaham, Y Toward a model of drug relapse: an assessment of the validity of the reinstatement procedure. *Psychopharmacology (Berl)* 2006; **189:** 1-16.

181. Ershler, WB, Keller, ET Age-associated increased interleukin-6 gene expression, late-life diseases, and frailty. *Annu Rev Med* 2000; **51:** 245-70.

182. Esposito, P, Chandler, N, Kandere, K, Basu, S, Jacobson, S, Connolly, R, Tutor, D, Theoharides, TC Corticotropin-releasing hormone and brain mast cells regulate blood-brain-barrier permeability induced by acute stress. *J Pharmacol Exp Ther* 2002; **303:** 1061-66.

183. Fadok, VA, Bratton, DL, Rose, DM, Pearson, A, Ezekewitz, RA, Henson, PM A receptor for phosphatidylserine-specific clearance of apoptotic cells. *Nature* 2000; **405:** 85-90.

184. Farah, A Interferon-induced depression treated with citalopram. *J Clin Psychiatry* 2002; **63:** 166-67.

185. Farooq, RK, Isingrini, E, Tanti, A, Le Guisquet, AM, Arlicot, N, Minier, F, Leman, S, Chalon, S, Belzung, C, Camus, V Is unpredictable chronic mild stress (UCMS) a reliable model to study depression-induced neuroinflammation? *Behav Brain Res* 2012; **231:** 130-137.

186. Fernandez-Guasti, A Sex, stress, and mood disorders: at the intersection of adrenal and gonadal hormones. 2012.

187. Ferrari, D, Chiozzi, P, Falzoni, S, Dal, SM, Melchiorri, L, Baricordi, OR, Di, VF Extracellular ATP triggers IL-1 beta release by activating the purinergic P2Z receptor of human macrophages. *J Immunol* 1997a; **159:** 1451-58.

188. Ferrari, D, Chiozzi, P, Falzoni, S, Dal, SM, Melchiorri, L, Baricordi, OR, Di, VF Extracellular ATP triggers IL-1 beta release by activating the purinergic P2Z receptor of human macrophages. *J Immunol* 1997b; **159:** 1451-58.

189. Ferrari, D, Chiozzi, P, Falzoni, S, Hanau, S, Di, VF Purinergic modulation of interleukin-1 beta release from microglial cells stimulated with bacterial endotoxin. *J Exp Med* 1997c; **185:** 579-82.

190. Ferrari, D, Pizzirani, C, Adinolfi, E, Lemoli, RM, Curti, A, Idzko, M, Panther, E, Di, VF The P2X7 receptor: a key player in IL-1 processing and release. *J Immunol* 2006; **176:** 3877-83.

191. Ferrari, D, Villalba, M, Chiozzi, P, Falzoni, S, Ricciardi-Castagnoli, P, Di, VF Mouse microglial cells express a plasma membrane pore

gated by extracellular ATP. *J Immunol* 1996; **156:** 1531-39.

192. Fietta, P, Fietta, P, Delsante, G Psychiatric and neuropsychological manifestations of systemic lupus erythematosus. *Acta Biomed* 2011; **82:** 97-114.

193. Fiorentino, DF, Zlotnik, A, Mosmann, TR, Howard, M, O'Garra, A IL-10 inhibits cytokine production by activated macrophages. *J Immunol* 1991; **147:** 3815-22.

194. Foster, T, Gillespie, K, McClelland, R Mental disorders and suicide in Northern Ireland. *Br J Psychiatry* 1997; **170:** 447-52.

195. Fredholm, BB, Abbracchio, MP, Burnstock, G, Daly, JW, Harden, TK, Jacobson, KA, Leff, P, Williams, M Nomenclature and classification of purinoceptors. *Pharmacol Rev* 1994; **46:** 143-56.

196. Fukuda, M, Mikuni, M [Clinical application of near-infrared spectroscopy (NIRS) in psychiatry: the advanced medical technology for differential diagnosis of depressive state]. *Seishin Shinkeigaku Zasshi* 2012; **114:** 801-6.

197. Fuller, SJ, Stokes, L, Skarratt, KK, Gu, BJ, Wiley, JS Genetics of the P2X7 receptor and human disease. *Purinergic Signal* 2009; **5:** 257-62.

198. Gaspar, P, Cases, O, Maroteaux, L The developmental role of serotonin: news from mouse molecular genetics. *Nat Rev Neurosci* 2003; **4:** 1002-12.

199. Gaykema, RP, Goehler, LE, Hansen, MK, Maier, SF, Watkins, LR Subdiaphragmatic vagotomy blocks interleukin-1beta-induced fever but does not reduce IL-1beta levels in the circulation. *Auton Neurosci* 2000; **85:** 72-77.

200. Ghanizadeh, A, Nouri, SZ, Nabi, SS Psychiatric problems and suicidal behaviour in incarcerated adolescents in the Islamic Republic of Iran. *East Mediterr Health J* 2012; **18:** 311-17.

201. GIBBONS, JL, McHUGH, PR Plasma cortisol in depressive illness. *J Psychiatr Res* 1962; **1:** 162-71.

202. Gitlin, N Hepatitis B: diagnosis, prevention, and treatment. *Clin Chem* 1997; **43:** 1500-1506.

203. Giulian, D, Baker, TJ, Shih, LC, Lachman, LB Interleukin 1 of the central nervous system is produced by ameboid microglia. *J Exp Med* 1986; **164:** 594-604.

204. Godbout, JP, Chen, J, Abraham, J, Richwine, AF, Berg, BM, Kelley, KW, Johnson, RW Exaggerated neuroinflammation and sickness behavior in aged mice following activation of the peripheral innate immune system. *FASEB J* 2005; **19:** 1329-31.

205. Goldman, LS Successful treatment of interferon alfa-induced mood disorder with nortriptyline. *Psychosomatics* 1994; **35:** 412-13.

206. Gomes-Leal, W Microglial physiopathology: how to explain the dual role of microglia after acute neural disorders? *Brain Behav* 2012; **2:** 345-56.

207. Gonzalez, A, Jenkins, JM, Steiner, M, Fleming, AS The relation between early life adversity, cortisol awakening response and diurnal salivary cortisol levels in postpartum women. *Psychoneuroendocrinology* 2009; **34:** 76-86.

208. Goshen, I, Kreisel, T, Ben-Menachem-Zidon, O, Licht, T, Weidenfeld, J, Ben-Hur, T, Yirmiya, R Brain interleukin-1 mediates chronic stress-induced depression in mice via adrenocortical activation and hippocampal neurogenesis suppression. *Mol Psychiatry* 2008; **13:** 717-28.

209. Graeber, MB Changing face of microglia. *Science* 2010; **330:** 783-88.

210. Greenberg, PE, Kessler, RC, Birnbaum, HG, Leong, SA, Lowe, SW, Berglund, PA, Corey-Lisle, PK The economic burden of depression in the United States: how did it change between 1990 and 2000? *J Clin Psychiatry* 2003; **64:** 1465-75.

211. Greig, AV, Linge, C, Cambrey, A, Burnstock, G Purinergic receptors are part of a signaling system for keratinocyte proliferation, differentiation, and apoptosis in human fetal epidermis. *J Invest Dermatol* 2003; **121:** 1145-49.

212. Griffiths, RJ, Stam, EJ, Downs, JT, Otterness, IG ATP induces the release of IL-1 from LPS-primed cells in vivo. *J Immunol* 1995; **154:** 2821-28.

213. Gross, C, Zhuang, X, Stark, K, Ramboz, S, Oosting, R, Kirby, L, Santarelli, L, Beck, S, Hen, R Serotonin1A receptor acts during development to establish normal anxiety-like behaviour in the adult. *Nature* 2002; **416:** 396-400.

214. Haenisch, B, Herms, S, Mattheisen, M, Steffens, M, Breuer, R, Strohmaier, J, Degenhardt, F, Schmal, C, Lucae, S, Maier, W, Rietschel, M, Nothen, MM, Cichon, S Genome-wide association data provide further support for an association between 5-HTTLPR and major depressive disorder. *J Affect Disord* 2012.

215. Halligan, SL, Herbert, J, Goodyer, IM, Murray, L Exposure to postnatal depression predicts elevated cortisol in adolescent offspring. *Biol Psychiatry* 2004; **55:** 376-81.

216. Hannestad, J, DellaGioia, N, Bloch, M The effect of antidepressant medication treatment on serum levels of inflammatory cytokines: a meta-analysis. *Neuropsychopharmacology* 2011; **36:** 2452-59.

217. Harada, K, Hide, I, Seki, T, Tanaka, S, Nakata, Y, Sakai, N Extracellular ATP differentially modulates Toll-like receptor 4-mediated cell survival and death of microglia. *J Neurochem* 2011; **116:** 1138-47.

218. Hart, BL Biological basis of the behavior of sick animals. *Neurosci Biobehav Rev* 1988; **12:** 123-37.

219. Harvey, D, Smith, R, English, K, Mahon, B, Commins, S Interleukin-10 (IL-10) but not Lipopolysaccharide (LPS) produces increased motor activity and abnormal exploratory patterns while impairing spatial learning in Balb/c mice. *Physiol Behav* 2006; **87:** 842-47.

220. Hattori, M, Gouaux, E Molecular mechanism of ATP binding and ion channel activation in P2X receptors. *Nature* 2012; **485:** 207-12.

221. Hayashi, F, Smith, KD, Ozinsky, A, Hawn, TR, Yi, EC, Goodlett, DR, Eng, JK, Akira, S, Underhill, DM, Aderem, A The innate immune response to bacterial flagellin is mediated by Toll-like receptor 5. *Nature* 2001; **410:** 1099-103.

222. Hayley, S, Scharf, J, Anisman, H Central administration of murine interferon-alpha induces depressive-like behavioral, brain cytokine and neurochemical alterations in mice: A mini-review and original experiments. *Brain Behav Immun* 2012.

223. Heim, C, Mletzko, T, Purselle, D, Musselman, DL, Nemeroff, CB The dexamethasone/corticotropin-releasing factor test in men with major depression: role of childhood trauma. *Biol Psychiatry* 2008; **63:** 398-405.

224. Heim, C, Nemeroff, CB Neurobiology of early life stress: clinical studies. *Semin Clin Neuropsychiatry* 2002; **7:** 147-59.

225. Heim, C, Newport, DJ, Wagner, D, Wilcox, MM, Miller, AH, Nemeroff, CB The role of early adverse experience and adulthood stress in the prediction of neuroendocrine stress reactivity in women: a multiple regression analysis. *Depress Anxiety* 2002; **15:** 117-25.

226. Hejjas, K, Szekely, A, Domotor, E, Halmai, Z, Balogh, G, Schilling, B, Sarosi, A, Faludi, G, Sasvari-Szekely, M, Nemoda, Z Association between depression and the Gln460Arg polymorphism of P2RX7 gene: a dimensional approach. *Am J Med Genet B Neuropsychiatr Genet* 2009; **150B:** 295-99.

227. Hemmerle, AM, Herman, JP, Seroogy, KB Stress, depression and Parkinson's disease. *Exp Neurol* 2012; **233:** 79-86.

228. Herman, JP, Ostrander, MM, Mueller, NK, Figueiredo, H Limbic system mechanisms of stress regulation: hypothalamo-pituitary-adrenocortical axis. *Prog Neuropsychopharmacol Biol Psychiatry* 2005; **29:** 1201-13.

229. Heyes, MP, Saito, K, Markey, SP Human macrophages convert L-

tryptophan into the neurotoxin quinolinic acid. *Biochem J* 1992a; **283 (Pt 3):** 633-35.

230. Heyes, MP, Saito, K, Markey, SP Human macrophages convert L-tryptophan into the neurotoxin quinolinic acid. *Biochem J* 1992b; **283 (Pt 3):** 633-35.

231. Hinwood, M, Morandini, J, Day, TA, Walker, FR Evidence that microglia mediate the neurobiological effects of chronic psychological stress on the medial prefrontal cortex. *Cereb Cortex* 2012; **22:** 1442-54.

232. Hippisley-Cox, J, Fielding, K, Pringle, M Depression as a risk factor for ischaemic heart disease in men: population based case-control study. *BMJ* 1998; **316:** 1714-19.

233. Hodgkin, PD, Bond, MW, O'Garra, A, Frank, G, Lee, F, Coffman, RL, Zlotnik, A, Howard, M Identification of IL-6 as a T cell-derived factor that enhances the proliferative response of thymocytes to IL-4 and phorbol myristate acetate. *J Immunol* 1988; **141:** 151-57.

234. Holsboer, F, Barden, N Antidepressants and hypothalamic-pituitary-adrenocortical regulation. *Endocr Rev* 1996; **17:** 187-205.

235. Holsboer, F, Weber, MM In memoriam Manfred Bleuler (1903-1994). *J Psychiatr Res* 1995; **29:** 1-3.

236. Horsmans, Y Chronic hepatitis C, depression and interferon. *J Hepatol* 2005; **42:** 788-89.

237. HORWITZ, D, LOVENBERG, W, ENGELMAN, K, SJOERDSMA, A MONOAMINE OXIDASE INHIBITORS, TYRAMINE, AND CHESSE. *JAMA* 1964; **188:** 1108-10.

238. Howren, MB, Lamkin, DM, Suls, J Associations of depression with C-reactive protein, IL-1, and IL-6: a meta-analysis. *Psychosom Med* 2009; **71:** 171-86.

239. Huber, SA, Sakkinen, P, Conze, D, Hardin, N, Tracy, R Interleukin-6 exacerbates early atherosclerosis in mice. *Arterioscler Thromb Vasc Biol* 1999; **19:** 2364-67.

240. Hulshof, HJ, Novati, A, Sgoifo, A, Luiten, PG, den Boer, JA, Meerlo, P Maternal separation decreases adult hippocampal cell proliferation and impairs cognitive performance but has little effect on stress sensitivity and anxiety in adult Wistar rats. *Behav Brain Res* 2011; **216:** 552-60.

241. Husain, MM, McClintock, SM, Rush, AJ, Knapp, RG, Fink, M, Rummans, TA, Rasmussen, K, Claassen, C, Petrides, G, Biggs, MM, Mueller, M, Sampson, S, Bailine, SH, Lisanby, SH, Kellner, CH The efficacy of acute electroconvulsive therapy in atypical depression. *J Clin Psychiatry* 2008; **69:** 406-11.

242. Husemann, J, Loike, JD, Anankov, R, Febbraio, M, Silverstein, SC Scavenger receptors in neurobiology and neuropathology: their role on microglia and other cells of the nervous system. *Glia* 2002; **40:** 195-205.

243. Idzko, M, Hammad, H, van, NM, Kool, M, Willart, MA, Muskens, F, Hoogsteden, HC, Luttmann, W, Ferrari, D, Di, VF, Virchow, JC, Jr., Lambrecht, BN Extracellular ATP triggers and maintains asthmatic airway inflammation by activating dendritic cells. *Nat Med* 2007; **13:** 913-19.

244. Iosif, RE, Ekdahl, CT, Ahlenius, H, Pronk, CJ, Bonde, S, Kokaia, Z, Jacobsen, SE, Lindvall, O Tumor necrosis factor receptor 1 is a negative regulator of progenitor proliferation in adult hippocampal neurogenesis. *J Neurosci* 2006; **26:** 9703-12.

245. Ishii, K, Kaneda, M, Li, H, Rockland, KS, Hashikawa, T Neuron-specific distribution of P2X7 purinergic receptors in the monkey retina. *J Comp Neurol* 2003; **459:** 267-77.

246. Isingrini, E, Camus, V, Le Guisquet, AM, Pingaud, M, Devers, S, Belzung, C Association between repeated unpredictable chronic mild stress (UCMS) procedures with a high fat diet: a model of fluoxetine resistance in mice. *PLoS One* 2010; **5:** e10404.

247. Isingrini, E, Desmidt, T, Belzung, C, Camus, V Endothelial dysfunction: A potential therapeutic target for geriatric depression and brain amyloid deposition in Alzheimer's disease? *Curr Opin Investig Drugs* 2009; **10:** 46-55.

248. Isingrini, E, Surget, A, Belzung, C, Freslon, JL, Frisbee, J, O'Donnell, J, Camus, V, d'Audiffret, A Altered aortic vascular reactivity in the unpredictable chronic mild stress model of depression in mice: UCMS causes relaxation impairment to ACh. *Physiol Behav* 2011; **103:** 540-546.

249. Isometsa, E, Henriksson, M, Marttunen, M, Heikkinen, M, Aro, H, Kuoppasalmi, K, Lonnqvist, J Mental disorders in young and middle aged men who commit suicide. *BMJ* 1995; **310:** 1366-67.

250. Jacob, KS, Bhugra, D, Lloyd, KR, Mann, AH Common mental disorders, explanatory models and consultation behaviour among Indian women living in the UK. *J R Soc Med* 1998; **91:** 66-71.

251. Jandl, M, Steyer, J, Kaschka, WP Suicide risk markers in major depressive disorder: a study of electrodermal activity and event-related potentials. *J Affect Disord* 2010; **123:** 138-49.

252. Jiang, LH, Mackenzie, AB, North, RA, Surprenant, A Brilliant blue G selectively blocks ATP-gated rat P2X(7) receptors. *Mol Pharmacol* 2000; **58:** 82-88.

253. Johnson, JD, Campisi, J, Sharkey, CM, Kennedy, SL, Nickerson, M, Greenwood, BN, Fleshner, M Catecholamines mediate stress-

induced increases in peripheral and central inflammatory cytokines. *Neuroscience* 2005; **135:** 1295-307.

254. Joseph, SM, Buchakjian, MR, Dubyak, GR Colocalization of ATP release sites and ecto-ATPase activity at the extracellular surface of human astrocytes. *J Biol Chem* 2003; **278:** 23331-42.

255. Kaczmarek-Hajek, K, Lorinczi, E, Hausmann, R, Nicke, A Molecular and functional properties of P2X receptors--recent progress and persisting challenges. *Purinergic Signal* 2012; **8:** 375-417.

256. Kahlenberg, JM, Dubyak, GR Mechanisms of caspase-1 activation by P2X7 receptor-mediated K+ release. *Am J Physiol Cell Physiol* 2004; **286:** C1100-C1108.

257. Kamaldeep BHUI Common mental disorders among people with origins in or immigrant from India and Pakistan. *International Review of Psychiatry* 1999; **11:** 136-44.

258. Kang, A, Hao, H, Zheng, X, Liang, Y, Xie, Y, Xie, T, Dai, C, Zhao, Q, Wu, X, Xie, L, Wang, G Peripheral anti-inflammatory effects explain the ginsenosides paradox between poor brain distribution and anti-depression efficacy. *J Neuroinflammation* 2011; **8:** 100.

259. Kasper, S The rationale for long-term antidepressant therapy. *Int Clin Psychopharmacol* 1993; **8:** 225-35.

260. Kasper, S, McEwen, BS Neurobiological and clinical effects of the antidepressant tianeptine. *CNS Drugs* 2008; **22:** 15-26.

261. Kastenschmidt, EK, Kennedy, GJ Depression and anxiety in late life: diagnostic insights and therapeutic options. *Mt Sinai J Med* 2011; **78:** 527-45.

262. Kaster, MP, Gadotti, VM, Calixto, JB, Santos, AR, Rodrigues, AL Depressive-like behavior induced by tumor necrosis factor-alpha in mice. *Neuropharmacology* 2012; **62:** 419-26.

263. Kato, T Molecular genetics of bipolar disorder and depression. *Psychiatry Clin Neurosci* 2007; **61:** 3-19.

264. Kaur, C, Hao, AJ, Wu, CH, Ling, EA Origin of microglia. *Microsc Res Tech* 2001; **54:** 2-9.

265. Kawai, T, Adachi, O, Ogawa, T, Takeda, K, Akira, S Unresponsiveness of MyD88-deficient mice to endotoxin. *Immunity* 1999; **11:** 115-22.

266. Kawai, T, Akira, S Signaling to NF-kappaB by Toll-like receptors. *Trends Mol Med* 2007; **13:** 460-469.

267. Kawakami, N, Takatsuka, N, Shimizu, H, Ishibashi, H Depressive symptoms and occurrence of type 2 diabetes among Japanese men. *Diabetes Care* 1999; **22:** 1071-76.

268. Keller, MB, McCullough, JP, Klein, DN, Arnow, B, Dunner, DL, Gelenberg, AJ, Markowitz, JC, Nemeroff, CB, Russell, JM, Thase, ME, Trivedi, MH, Zajecka, J A comparison of nefazodone, the cognitive behavioral-analysis system of psychotherapy, and their combination for the treatment of chronic depression. *N Engl J Med* 2000; **342:** 1462-70.

269. Kelly, WF Psychiatric aspects of Cushing's syndrome. *QJM* 1996; **89:** 543-51.

270. Kempermann, G, Neumann, H Neuroscience. Microglia: the enemy within? *Science* 2003; **302:** 1689-90.

271. Kendal, WS Suicide and cancer: a gender-comparative study. *Ann Oncol* 2007; **18:** 381-87.

272. Kendler, KS, Gardner, CO, Prescott, CA Clinical characteristics of major depression that predict risk of depression in relatives. *Arch Gen Psychiatry* 1999; **56:** 322-27.

273. Kendler, KS, Gatz, M, Gardner, CO, Pedersen, NL A Swedish national twin study of lifetime major depression. *Am J Psychiatry* 2006; **163:** 109-14.

274. Kent, S, Bluthe, RM, Dantzer, R, Hardwick, AJ, Kelley, KW, Rothwell, NJ, Vannice, JL Different receptor mechanisms mediate the pyrogenic and behavioral effects of interleukin 1. *Proc Natl Acad Sci U S A* 1992a; **89:** 9117-20.

275. Kent, S, Bluthe, RM, Dantzer, R, Hardwick, AJ, Kelley, KW, Rothwell, NJ, Vannice, JL Different receptor mechanisms mediate the pyrogenic and behavioral effects of interleukin 1. *Proc Natl Acad Sci U S A* 1992b; **89:** 9117-20.

276. Kessler, RC, Birnbaum, HG, Shahly, V, Bromet, E, Hwang, I, McLaughlin, KA, Sampson, N, Andrade, LH, de, GG, Demyttenaere, K, Haro, JM, Karam, AN, Kostyuchenko, S, Kovess, V, Lara, C, Levinson, D, Matschinger, H, Nakane, Y, Browne, MO, Ormel, J, Posada-Villa, J, Sagar, R, Stein, DJ Age differences in the prevalence and co-morbidity of DSM-IV major depressive episodes: results from the WHO World Mental Health Survey Initiative. *Depress Anxiety* 2010a; **27:** 351-64.

277. Kessler, RC, Birnbaum, HG, Shahly, V, Bromet, E, Hwang, I, McLaughlin, KA, Sampson, N, Andrade, LH, de, GG, Demyttenaere, K, Haro, JM, Karam, AN, Kostyuchenko, S, Kovess, V, Lara, C, Levinson, D, Matschinger, H, Nakane, Y, Browne, MO, Ormel, J, Posada-Villa, J, Sagar, R, Stein, DJ Age differences in the prevalence and co-morbidity of DSM-IV major depressive episodes: results from the WHO World Mental Health Survey Initiative. *Depress Anxiety* 2010b; **27:** 351-64.

278. Kessler, RC, McGonagle, KA, Swartz, M, Blazer, DG, Nelson, CB Sex and depression in the National Comorbidity Survey. I: Lifetime

prevalence, chronicity and recurrence. *J Affect Disord* 1993a; **29:** 85-96.

279. Kessler, RC, McGonagle, KA, Swartz, M, Blazer, DG, Nelson, CB Sex and depression in the National Comorbidity Survey. I: Lifetime prevalence, chronicity and recurrence. *J Affect Disord* 1993b; **29:** 85-96.

280. Kessler, RC, Merikangas, KR, Wang, PS Prevalence, comorbidity, and service utilization for mood disorders in the United States at the beginning of the twenty-first century. *Annu Rev Clin Psychol* 2007; **3:** 137-58.

281. Khakh, BS Molecular physiology of P2X receptors and ATP signalling at synapses. *Nat Rev Neurosci* 2001; **2:** 165-74.

282. Kho, KH, van Vreeswijk, MF, Simpson, S, Zwinderman, AH A meta-analysis of electroconvulsive therapy efficacy in depression. *J ECT* 2003; **19:** 139-47.

283. Kinder, LS, Kamarck, TW, Baum, A, Orchard, TJ Depressive symptomatology and coronary heart disease in Type I diabetes mellitus: a study of possible mechanisms. *Health Psychol* 2002; **21:** 542-52.

284. Kluger, MJ Fever: role of pyrogens and cryogens. *Physiol Rev* 1991; **71:** 93-127.

285. Kluger, MJ, Kozak, W, Conn, CA, Leon, LR, Soszynski, D Role of fever in disease. *Ann N Y Acad Sci* 1998; **856:** 224-33.

286. Koedel, U, Bernatowicz, A, Frei, K, Fontana, A, Pfister, HW Systemically (but not intrathecally) administered IL-10 attenuates pathophysiologic alterations in experimental pneumococcal meningitis. *J Immunol* 1996; **157:** 5185-91.

287. Koizumi, S, Shigemoto-Mogami, Y, Nasu-Tada, K, Shinozaki, Y, Ohsawa, K, Tsuda, M, Joshi, BV, Jacobson, KA, Kohsaka, S, Inoue, K UDP acting at P2Y6 receptors is a mediator of microglial phagocytosis. *Nature* 2007; **446:** 1091-95.

288. Koj, A, Magielska-Zero, D, Bereta, J, Kurdowska, A, Rokita, H, Gauldie, J The cascade of inflammatory cytokines regulating synthesis of acute phase proteins. *Tokai J Exp Clin Med* 1988; **13:** 255-64.

289. Kontoangelos, K, Raptis, AE, Papageorgiou, CC, Papadimitriou, GN, Rabavilas, AD, Dimitriadis, G, Raptis, SA The association of the metabolic profile in diabetes mellitus type 2 patients with obsessive-compulsive symptomatology and depressive symptomatology: New insights. *Int J Psychiatry Clin Pract* 2012.

290. Koo, JW, Duman, RS IL-1beta is an essential mediator of the antineurogenic and anhedonic effects of stress. *Proc Natl Acad Sci U*

S A 2008; **105:** 751-56.

291. Koshimizu, TA, Van, GF, Tomic, M, Wong, AO, Tanoue, A, Tsujimoto, G, Stojilkovic, SS Characterization of calcium signaling by purinergic receptor-channels expressed in excitable cells. *Mol Pharmacol* 2000; **58:** 936-45.

292. Kreutzberg, GW Microglia: a sensor for pathological events in the CNS. *Trends Neurosci* 1996; **19:** 312-18.

293. Krishnadas, R, Cavanagh, J Depression: an inflammatory illness? *J Neurol Neurosurg Psychiatry* 2012; **83:** 495-502.

294. Krishnan, V, Nestler, EJ The molecular neurobiology of depression. *Nature* 2008; **455:** 894-902.

295. Kronfol, Z, House, JD Lymphocyte mitogenesis, immunoglobulin and complement levels in depressed patients and normal controls. *Acta Psychiatr Scand* 1989; **80:** 142-47.

296. Krueger, JM, Taishi, P, De, A, Davis, CJ, Winters, BD, Clinton, J, Szentirmai, E, Zielinski, MR ATP and the purine type 2 X7 receptor affect sleep. *J Appl Physiol* 2010; **109:** 1318-27.

297. Krysko, DV, Vanden Berghe, T, D'Herde, K, Vandenabeele, P Apoptosis and necrosis: detection, discrimination and phagocytosis. *Methods* 2008; **44:** 205-21.

298. Kubera, M, Lin, AH, Kenis, G, Bosmans, E, van, BD, Maes, M Anti-Inflammatory effects of antidepressants through suppression of the interferon-gamma/interleukin-10 production ratio. *J Clin Psychopharmacol* 2001; **21:** 199-206.

299. Kumari, M, Head, J, Bartley, M, Stansfeld, S, Kivimaki, M Maternal separation in childhood and diurnal cortisol patterns in mid-life: findings from the Whitehall II study. *Psychol Med* 2012; 1-11.

300. Kunugi, H, Ida, I, Owashi, T, Kimura, M, Inoue, Y, Nakagawa, S, Yabana, T, Urushibara, T, Kanai, R, Aihara, M, Yuuki, N, Otsubo, T, Oshima, A, Kudo, K, Inoue, T, Kitaichi, Y, Shirakawa, O, Isogawa, K, Nagayama, H, Kamijima, K, Nanko, S, Kanba, S, Higuchi, T, Mikuni, M Assessment of the dexamethasone/CRH test as a state-dependent marker for hypothalamic-pituitary-adrenal (HPA) axis abnormalities in major depressive episode: a Multicenter Study. *Neuropsychopharmacology* 2006; **31:** 212-20.

301. Kwidzinski, E, Bechmann, I IDO expression in the brain: a double-edged sword. *J Mol Med (Berl)* 2007; **85:** 1351-59.

302. Labrousse, VF, Costes, L, Aubert, A, Darnaudery, M, Ferreira, G, Amedee, T, Laye, S Impaired interleukin-1beta and c-Fos expression in the hippocampus is associated with a spatial memory deficit in P2X(7) receptor-deficient mice. *PLoS One* 2009; **4:** e6006.

303. Lajud, N, Roque, A, Cajero, M, Gutierrez-Ospina, G, Torner, L Periodic maternal separation decreases hippocampal neurogenesis without affecting basal corticosterone during the stress hyporesponsive period, but alters HPA axis and coping behavior in adulthood. *Psychoneuroendocrinology* 2012; **37:** 410-420.

304. Lanquillon, S, Krieg, JC, Bening-Abu-Shach, U, Vedder, H Cytokine production and treatment response in major depressive disorder. *Neuropsychopharmacology* 2000a; **22:** 370-379.

305. Lanquillon, S, Krieg, JC, Bening-Abu-Shach, U, Vedder, H Cytokine production and treatment response in major depressive disorder. *Neuropsychopharmacology* 2000b; **22:** 370-379.

306. Lauber, K, Blumenthal, SG, Waibel, M, Wesselborg, S Clearance of apoptotic cells: getting rid of the corpses. *Mol Cell* 2004; **14:** 277-87.

307. Laucht, M, Treutlein, J, Blomeyer, D, Buchmann, AF, Schmidt, MH, Esser, G, Jennen-Steinmetz, C, Rietschel, M, Banaschewski, T Interactive effects of corticotropin-releasing hormone receptor 1 gene and childhood adversity on depressive symptoms in young adults: Findings from a longitudinal study. *Eur Neuropsychopharmacol* 2012.

308. Laugeray, A, Launay, JM, Callebert, J, Surget, A, Belzung, C, Barone, PR Peripheral and cerebral metabolic abnormalities of the tryptophan-kynurenine pathway in a murine model of major depression. *Behav Brain Res* 2010; **210:** 84-91.

309. Ledeboer, A, Breve, JJ, Poole, S, Tilders, FJ, Van Dam, AM Interleukin-10, interleukin-4, and transforming growth factor-beta differentially regulate lipopolysaccharide-induced production of pro-inflammatory cytokines and nitric oxide in co-cultures of rat astroglial and microglial cells. *Glia* 2000; **30:** 134-42.

310. Ledeboer, A, Breve, JJ, Wierinckx, A, van der Jagt, S, Bristow, AF, Leysen, JE, Tilders, FJ, Van Dam, AM Expression and regulation of interleukin-10 and interleukin-10 receptor in rat astroglial and microglial cells. *Eur J Neurosci* 2002; **16:** 1175-85.

311. Lee, J, Duan, W, Mattson, MP Evidence that brain-derived neurotrophic factor is required for basal neurogenesis and mediates, in part, the enhancement of neurogenesis by dietary restriction in the hippocampus of adult mice. *J Neurochem* 2002; **82:** 1367-75.

312. Lee, S, Jeong, J, Kwak, Y, Park, SK Depression research: where are we now? *Mol Brain* 2010; **3:** 8.

313. Lees, MP, Fuller, SJ, McLeod, R, Boulter, NR, Miller, CM, Zakrzewski, AM, Mui, EJ, Witola, WH, Coyne, JJ, Hargrave, AC, Jamieson, SE, Blackwell, JM, Wiley, JS, Smith, NC P2X7 receptor-mediated killing of an intracellular parasite, Toxoplasma gondii, by human and

murine macrophages. *J Immunol* 2010; **184:** 7040-7046.

314. Lemoine, M How does a psychiatrist infer from an observed condition to a case of mental disorder? *J Eval Clin Pract* 2012; **18:** 979-83.

315. Lemstra, AW, Groen in't Woud, JC, Hoozemans, JJ, van Haastert, ES, Rozemuller, AJ, Eikelenboom, P, van Gool, WA Microglia activation in sepsis: a case-control study. *J Neuroinflammation* 2007; **4:** 4.

316. Leon, LR, Kozak, W, Rudolph, K, Kluger, MJ An antipyretic role for interleukin-10 in LPS fever in mice. *Am J Physiol* 1999; **276:** R81-R89.

317. Lesch, KP, Bengel, D, Heils, A, Sabol, SZ, Greenberg, BD, Petri, S, Benjamin, J, Muller, CR, Hamer, DH, Murphy, DL Association of anxiety-related traits with a polymorphism in the serotonin transporter gene regulatory region. *Science* 1996; **274:** 1527-31.

318. Levine, J, Barak, Y, Chengappa, KN, Rapoport, A, Rebey, M, Barak, V Cerebrospinal cytokine levels in patients with acute depression. *Neuropsychobiology* 1999; **40:** 171-76.

319. Lewinsohn, PM, Rohde, P, Seeley, JR Major depressive disorder in older adolescents: prevalence, risk factors, and clinical implications. *Clin Psychol Rev* 1998; **18:** 765-94.

320. Li, C, Zienkiewicz, J, Hawiger, J Interactive sites in the MyD88 Toll/interleukin (IL) 1 receptor domain responsible for coupling to the IL1beta signaling pathway. *J Biol Chem* 2005; **280:** 26152-59.

321. Li, P, Allen, H, Banerjee, S, Franklin, S, Herzog, L, Johnston, C, McDowell, J, Paskind, M, Rodman, L, Salfeld, J, . Mice deficient in IL-1 beta-converting enzyme are defective in production of mature IL-1 beta and resistant to endotoxic shock. *Cell* 1995; **80:** 401-11.

322. Li, Q, Luo, X, Muallem, S Regulation of the P2X7 receptor permeability to large molecules by extracellular Cl- and Na+. *J Biol Chem* 2005; **280:** 26922-27.

323. Lin, EH, Rutter, CM, Katon, W, Heckbert, SR, Ciechanowski, P, Oliver, MM, Ludman, EJ, Young, BA, Williams, LH, McCulloch, DK, Von, KM Depression and advanced complications of diabetes: a prospective cohort study. *Diabetes Care* 2010; **33:** 264-69.

324. Lindemann, RA The regulatory effects of monocytes on human natural killer cells activated by lipopolysaccharides. *J Periodontal Res* 1991; **26:** 486-90.

325. LINGJAERDE, O TETRABENAZINE (NITOMAN) IN THE TREATMENT OF PSYCHOSES. WITH A DISCUSSION ON THE CENTRAL MODE OF ACTION OF TETRABENAZINE AND RESERPINE. *Acta Psychiatr Scand* 1963; **39:** SUPPL170-109.

326. Liu, YP, Lin, HI, Tzeng, SF Tumor necrosis factor-alpha and interleukin-18 modulate neuronal cell fate in embryonic neural progenitor culture. *Brain Res* 2005; **1054:** 152-58.

327. Lotrich, FE, Rabinovitz, M, Gironda, P, Pollock, BG Depression following pegylated interferon-alpha: characteristics and vulnerability. *J Psychosom Res* 2007; **63:** 131-35.

328. Lotufo-Neto, F, Trivedi, M, Thase, ME Meta-analysis of the reversible inhibitors of monoamine oxidase type A moclobemide and brofaromine for the treatment of depression. *Neuropsychopharmacology* 1999; **20:** 226-47.

329. Lucae, S, Salyakina, D, Barden, N, Harvey, M, Gagne, B, Labbe, M, Binder, EB, Uhr, M, Paez-Pereda, M, Sillaber, I, Ising, M, Bruckl, T, Lieb, R, Holsboer, F, Muller-Myhsok, B P2RX7, a gene coding for a purinergic ligand-gated ion channel, is associated with major depressive disorder. *Hum Mol Genet* 2006; **15:** 2438-45.

330. Luheshi, GN, Bluthe, RM, Rushforth, D, Mulcahy, N, Konsman, JP, Goldbach, M, Dantzer, R Vagotomy attenuates the behavioural but not the pyrogenic effects of interleukin-1 in rats. *Auton Neurosci* 2000; **85:** 127-32.

331. Lytinas, M, Kempuraj, D, Huang, M, Boucher, W, Esposito, P, Theoharides, TC Acute stress results in skin corticotropin-releasing hormone secretion, mast cell activation and vascular permeability, an effect mimicked by intradermal corticotropin-releasing hormone and inhibited by histamine-1 receptor antagonists. *Int Arch Allergy Immunol* 2003; **130:** 224-31.

332. Mackay, GM, Forrest, CM, Christofides, J, Bridel, MA, Mitchell, S, Cowlard, R, Stone, TW, Darlington, LG Kynurenine metabolites and inflammation markers in depressed patients treated with fluoxetine or counselling. *Clin Exp Pharmacol Physiol* 2009; **36:** 425-35.

333. MacQueen, G, Frodl, T The hippocampus in major depression: evidence for the convergence of the bench and bedside in psychiatric research? *Mol Psychiatry* 2011; **16:** 252-64.

334. Maelfait, J, Vercammen, E, Janssens, S, Schotte, P, Haegman, M, Magez, S, Beyaert, R Stimulation of Toll-like receptor 3 and 4 induces interleukin-1beta maturation by caspase-8. *J Exp Med* 2008; **205:** 1967-73.

335. Maes, M, Bosmans, E, Meltzer, HY Immunoendocrine aspects of major depression. Relationships between plasma interleukin-6 and soluble interleukin-2 receptor, prolactin and cortisol. *Eur Arch Psychiatry Clin Neurosci* 1995; **245:** 172-78.

336. Maes, M, Bosmans, E, Suy, E, Vandervorst, C, DeJonckheere, C, Raus, J Depression-related disturbances in mitogen-induced lymphocyte responses and interleukin-1 beta and soluble interleukin-2

receptor production. *Acta Psychiatr Scand* 1991; **84:** 379-86.

337. Maes, M, Mihaylova, I, Kubera, M, Ringel, K Activation of cell-mediated immunity in depression: association with inflammation, melancholia, clinical staging and the fatigue and somatic symptom cluster of depression. *Prog Neuropsychopharmacol Biol Psychiatry* 2012; **36:** 169-75.

338. Maes, M, Van, d, V, Vandoolaeghe, E, Bril, T, Demedts, P, Wauters, A, Neels, H Alterations in iron metabolism and the erythron in major depression: further evidence for a chronic inflammatory process. *J Affect Disord* 1996; **40:** 23-33.

339. Magarinos, AM, Orchinik, M, McEwen, BS Morphological changes in the hippocampal CA3 region induced by non-invasive glucocorticoid administration: a paradox. *Brain Res* 1998; **809:** 314-18.

340. Malberg, JE, Eisch, AJ, Nestler, EJ, Duman, RS Chronic antidepressant treatment increases neurogenesis in adult rat hippocampus. *J Neurosci* 2000; **20:** 9104-10.

341. Malkesman, O, Braw, Y, Ram, E, Maayan, R, Weizman, A, Kinor, N, Yadid, G, Weller, A Dehydroepiandrosterone and monoamines in the limbic system of a genetic animal model of childhood depression. *Eur Neuropsychopharmacol* 2008; **18:** 255-61.

342. Mandrioli, R, Mercolini, L, Saracino, MA, Raggi, MA Selective serotonin reuptake inhibitors (SSRIs): therapeutic drug monitoring and pharmacological interactions. *Curr Med Chem* 2012; **19:** 1846-63.

343. Marangell, LB Switching antidepressants for treatment-resistant major depression. *J Clin Psychiatry* 2001; **62 Suppl 18:** 12-17.

344. Marsh, CB, Moore, SA, Pope, HA, Wewers, MD IL-1ra suppresses endotoxin-induced IL-1 beta and TNF-alpha release from mononuclear phagocytes. *Am J Physiol* 1994; **267:** L39-L45.

345. Marsland, AL, Petersen, KL, Sathanoori, R, Muldoon, MF, Neumann, SA, Ryan, C, Flory, JD, Manuck, SB Interleukin-6 covaries inversely with cognitive performance among middle-aged community volunteers. *Psychosom Med* 2006; **68:** 895-903.

346. Martinon, F, Tschopp, J Inflammatory caspases and inflammasomes: master switches of inflammation. *Cell Death Differ* 2007; **14:** 10-22.

347. Massart, R, Mongeau, R, Lanfumey, L Beyond the monoaminergic hypothesis: neuroplasticity and epigenetic changes in a transgenic mouse model of depression. *Philos Trans R Soc Lond B Biol Sci* 2012; **367:** 2485-94.

348. Mayer, P, Geissler, K, Valent, P, Ceska, M, Bettelheim, P, Liehl, E Recombinant human interleukin 6 is a potent inducer of the acute

phase response and elevates the blood platelets in nonhuman primates. *Exp Hematol* 1991; **19:** 688-96.

349. Mazure C.M. Life Stressors as Risk Factors in Depression. *Clinical Psychology: Science and Practice* 1998; **5:** 291-313.

350. Mazure, CM, Bruce, ML, Maciejewski, PK, Jacobs, SC Adverse life events and cognitive-personality characteristics in the prediction of major depression and antidepressant response. *Am J Psychiatry* 2000; **157:** 896-903.

351. McArthur, S, Cristante, E, Paterno, M, Christian, H, Roncaroli, F, Gillies, GE, Solito, E Annexin A1: a central player in the anti-inflammatory and neuroprotective role of microglia. *J Immunol* 2010; **185:** 6317-28.

352. McGeer, PL, McGeer, EG Glial cell reactions in neurodegenerative diseases: pathophysiology and therapeutic interventions. *Alzheimer Dis Assoc Disord* 1998; **12 Suppl 2:** S1-S6.

353. McLarnon, JG, Ryu, JK, Walker, DG, Choi, HB Upregulated expression of purinergic P2X(7) receptor in Alzheimer disease and amyloid-beta peptide-treated microglia and in peptide-injected rat hippocampus. *J Neuropathol Exp Neurol* 2006a; **65:** 1090-1097.

354. McLarnon, JG, Ryu, JK, Walker, DG, Choi, HB Upregulated expression of purinergic P2X(7) receptor in Alzheimer disease and amyloid-beta peptide-treated microglia and in peptide-injected rat hippocampus. *J Neuropathol Exp Neurol* 2006b; **65:** 1090-1097.

355. McMahon, FJ, Buervenich, S, Charney, D, Lipsky, R, Rush, AJ, Wilson, AF, Sorant, AJ, Papanicolaou, GJ, Laje, G, Fava, M, Trivedi, MH, Wisniewski, SR, Manji, H Variation in the gene encoding the serotonin 2A receptor is associated with outcome of antidepressant treatment. *Am J Hum Genet* 2006; **78:** 804-14.

356. Mellor, AL, Munn, DH Tryptophan catabolism and T-cell tolerance: immunosuppression by starvation? *Immunol Today* 1999; **20:** 469-73.

357. Mendlewicz, J, Kriwin, P, Oswald, P, Souery, D, Alboni, S, Brunello, N Shortened onset of action of antidepressants in major depression using acetylsalicylic acid augmentation: a pilot open-label study. *Int Clin Psychopharmacol* 2006; **21:** 227-31.

358. Mesquita, AR, Correia-Neves, M, Roque, S, Castro, AG, Vieira, P, Pedrosa, J, Palha, JA, Sousa, N IL-10 modulates depressive-like behavior. *J Psychiatr Res* 2008a; **43:** 89-97.

359. Mesquita, AR, Correia-Neves, M, Roque, S, Castro, AG, Vieira, P, Pedrosa, J, Palha, JA, Sousa, N IL-10 modulates depressive-like behavior. *J Psychiatr Res* 2008b; **43:** 89-97.

360. Mesquita, AR, Correia-Neves, M, Roque, S, Castro, AG, Vieira, P,

Pedrosa, J, Palha, JA, Sousa, N IL-10 modulates depressive-like behavior. *J Psychiatr Res* 2008c; **43:** 89-97.

361. Meyer, JH, Ginovart, N, Boovariwala, A, Sagrati, S, Hussey, D, Garcia, A, Young, T, Praschak-Rieder, N, Wilson, AA, Houle, S Elevated monoamine oxidase a levels in the brain: an explanation for the monoamine imbalance of major depression. *Arch Gen Psychiatry* 2006; **63:** 1209-16.

362. Mikova, O, Yakimova, R, Bosmans, E, Kenis, G, Maes, M Increased serum tumor necrosis factor alpha concentrations in major depression and multiple sclerosis. *Eur Neuropsychopharmacol* 2001; **11:** 203-8.

363. Mill, J, Petronis, A Molecular studies of major depressive disorder: the epigenetic perspective. *Mol Psychiatry* 2007; **12:** 799-814.

364. Miller, AH, Maletic, V, Raison, CL Inflammation and its discontents: the role of cytokines in the pathophysiology of major depression. *Biol Psychiatry* 2009; **65:** 732-41.

365. Miller, AH, Raison, CL Cytokines, p38 MAP kinase and the pathophysiology of depression. *Neuropsychopharmacology* 2006; **31:** 2089-90.

366. Moller, SE, Kirk, L, Honore, P Tryptophan tolerance and metabolism in endogenous depression. *Psychopharmacology (Berl)* 1982; **76:** 79-83.

367. Monif, M, Burnstock, G, Williams, DA Microglia: proliferation and activation driven by the P2X7 receptor. *Int J Biochem Cell Biol* 2010; **42:** 1753-56.

368. Monje, ML, Toda, H, Palmer, TD Inflammatory blockade restores adult hippocampal neurogenesis. *Science* 2003a; **302:** 1760-1765.

369. Monje, ML, Toda, H, Palmer, TD Inflammatory blockade restores adult hippocampal neurogenesis. *Science* 2003b; **302:** 1760-1765.

370. Monkul, ES, Hatch, JP, Nicoletti, MA, Spence, S, Brambilla, P, Lacerda, AL, Sassi, RB, Mallinger, AG, Keshavan, MS, Soares, JC Fronto-limbic brain structures in suicidal and non-suicidal female patients with major depressive disorder. *Mol Psychiatry* 2007; **12:** 360-366.

371. Monroe, SM, Harkness, K, Simons, AD, Thase, ME Life stress and the symptoms of major depression. *J Nerv Ment Dis* 2001; **189:** 168-75.

372. Monroe, SM, Simons, AD Diathesis-stress theories in the context of life stress research: implications for the depressive disorders. *Psychol Bull* 1991; **110:** 406-25.

373. Montgomery, SA, Henry, J, McDonald, G, Dinan, T, Lader, M, Hindmarch, I, Clare, A, Nutt, D Selective serotonin reuptake

inhibitors: meta-analysis of discontinuation rates. *Int Clin Psychopharmacol* 1994; **9:** 47-53.

374. Moore, KW, de Waal, MR, Coffman, RL, O'Garra, A Interleukin-10 and the interleukin-10 receptor. *Annu Rev Immunol* 2001; **19:** 683-765.

375. Moores, TS, Hasdemir, B, Vega-Riveroll, L, Deuchars, J, Parson, SH Properties of presynaptic P2X7-like receptors at the neuromuscular junction. *Brain Res* 2005; **1034:** 40-50.

376. Moscicki, EK Gender differences in completed and attempted suicides. *Ann Epidemiol* 1994; **4:** 152-58.

377. Mueller, TI, Leon, AC, Keller, MB, Solomon, DA, Endicott, J, Coryell, W, Warshaw, M, Maser, JD Recurrence after recovery from major depressive disorder during 15 years of observational follow-up. *Am J Psychiatry* 1999a; **156:** 1000-1006.

378. Mueller, TI, Leon, AC, Keller, MB, Solomon, DA, Endicott, J, Coryell, W, Warshaw, M, Maser, JD Recurrence after recovery from major depressive disorder during 15 years of observational follow-up. *Am J Psychiatry* 1999b; **156:** 1000-1006.

379. Murgia, M, Pizzo, P, Steinberg, TH, Di, VF Characterization of the cytotoxic effect of extracellular ATP in J774 mouse macrophages. *Biochem J* 1992; **288 (Pt 3):** 897-901.

380. Murray, CA, Lynch, MA Evidence that increased hippocampal expression of the cytokine interleukin-1 beta is a common trigger for age- and stress-induced impairments in long-term potentiation. *J Neurosci* 1998; **18:** 2974-81.

381. Murrough, JW, Perez, AM, Pillemer, S, Stern, J, Parides, MK, Aan Het, RM, Collins, KA, Mathew, SJ, Charney, DS, Iosifescu, DV Rapid and Longer-Term Antidepressant Effects of Repeated Ketamine Infusions in Treatment-Resistant Major Depression. *Biol Psychiatry* 2012.

382. Musselman, DL, Lawson, DH, Gumnick, JF, Manatunga, AK, Penna, S, Goodkin, RS, Greiner, K, Nemeroff, CB, Miller, AH Paroxetine for the prevention of depression induced by high-dose interferon alfa. *N Engl J Med* 2001; **344:** 961-66.

383. Myint, AM, Kim, YK Cytokine-serotonin interaction through IDO: a neurodegeneration hypothesis of depression. *Med Hypotheses* 2003; **61:** 519-25.

384. Myint, AM, Kim, YK, Verkerk, R, Scharpe, S, Steinbusch, H, Leonard, B Kynurenine pathway in major depression: evidence of impaired neuroprotection. *J Affect Disord* 2007; **98:** 143-51.

385. Nakanishi, M, Niidome, T, Matsuda, S, Akaike, A, Kihara, T, Sugimoto, H Microglia-derived interleukin-6 and leukaemia

inhibitory factor promote astrocytic differentiation of neural stem/progenitor cells. *Eur J Neurosci* 2007; **25:** 649-58.

386. Nava, F, Calapai, G, Facciola, G, Cuzzocrea, S, Marciano, MC, De, SA, Caputi, AP Effects of interleukin-10 on water intake, locomotory activity, and rectal temperature in rat treated with endotoxin. *Int J Immunopharmacol* 1997; **19:** 31-38.

387. Neher, JJ, Neniskyte, U, Brown, GC Primary phagocytosis of neurons by inflamed microglia: potential roles in neurodegeneration. *Front Pharmacol* 2012; **3:** 27.

388. Neher, JJ, Neniskyte, U, Zhao, JW, Bal-Price, A, Tolkovsky, AM, Brown, GC Inhibition of microglial phagocytosis is sufficient to prevent inflammatory neuronal death. *J Immunol* 2011a; **186:** 4973-83.

389. Neher, JJ, Neniskyte, U, Zhao, JW, Bal-Price, A, Tolkovsky, AM, Brown, GC Inhibition of microglial phagocytosis is sufficient to prevent inflammatory neuronal death. *J Immunol* 2011b; **186:** 4973-83.

390. Nemeroff, CB Psychopharmacology of affective disorders in the 21st century. *Biol Psychiatry* 1998; **44:** 517-25.

391. Nemeroff, CB, Widerlov, E, Bissette, G, Walleus, H, Karlsson, I, Eklund, K, Kilts, CD, Loosen, PT, Vale, W Elevated concentrations of CSF corticotropin-releasing factor-like immunoreactivity in depressed patients. *Science* 1984; **226:** 1342-44.

392. Nery, FG, Hatch, JP, Nicoletti, MA, Monkul, ES, Najt, P, Matsuo, K, Cloninger, CR, Soares, JC Temperament and character traits in major depressive disorder: influence of mood state and recurrence of episodes. *Depress Anxiety* 2009; **26:** 382-88.

393. Nestler, EJ, Hyman, SE Animal models of neuropsychiatric disorders. *Nat Neurosci* 2010; **13:** 1161-69.

394. Nguyen, KT, Deak, T, Owens, SM, Kohno, T, Fleshner, M, Watkins, LR, Maier, SF Exposure to acute stress induces brain interleukin-1beta protein in the rat. *J Neurosci* 1998a; **18:** 2239-46.

395. Nguyen, KT, Deak, T, Owens, SM, Kohno, T, Fleshner, M, Watkins, LR, Maier, SF Exposure to acute stress induces brain interleukin-1beta protein in the rat. *J Neurosci* 1998b; **18:** 2239-46.

396. Nibuya, M, Morinobu, S, Duman, RS Regulation of BDNF and trkB mRNA in rat brain by chronic electroconvulsive seizure and antidepressant drug treatments. *J Neurosci* 1995; **15:** 7539-47.

397. Niederhofer, H [Therapy resistant major depression: improvement of symptomatology after combining antidepressants with Tianeptine (Stablon)]. *Psychiatr Prax* 2003; **30:** 221-22.

398. Nimmerjahn, A, Kirchhoff, F, Helmchen, F Resting microglial cells are highly dynamic surveillants of brain parenchyma in vivo. *Science* 2005; **308:** 1314-18.

399. Noble, RE Depression in women. *Metabolism* 2005a; **54:** 49-52.

400. Noble, RE Depression in women. *Metabolism* 2005b; **54:** 49-52.

401. Nolen-Hoeksema, S Sex differences in unipolar depression: evidence and theory. *Psychol Bull* 1987; **101:** 259-82.

402. North, RA Families of ion channels with two hydrophobic segments. *Curr Opin Cell Biol* 1996; **8:** 474-83.

403. North, RA Molecular physiology of P2X receptors. *Physiol Rev* 2002; **82:** 1013-67.

404. North, RA, Surprenant, A Pharmacology of cloned P2X receptors. *Annu Rev Pharmacol Toxicol* 2000; **40:** 563-80.

405. Nunes, SO, Vargas, HO, Brum, J, Prado, E, Vargas, MM, de Castro, MR, Dodd, S, Berk, M A comparison of inflammatory markers in depressed and nondepressed smokers. *Nicotine Tob Res* 2012; **14:** 540-546.

406. Ohira, T, Iso, H, Satoh, S, Sankai, T, Tanigawa, T, Ogawa, Y, Imano, H, Sato, S, Kitamura, A, Shimamoto, T Prospective study of depressive symptoms and risk of stroke among japanese. *Stroke* 2001; **32:** 903-8.

407. Olfson, M, Marcus, SC, Druss, BG Effects of Food and Drug Administration warnings on antidepressant use in a national sample. *Arch Gen Psychiatry* 2008; **65:** 94-101.

408. Olson, JK, Miller, SD Microglia initiate central nervous system innate and adaptive immune responses through multiple TLRs. *J Immunol* 2004; **173:** 3916-24.

409. Opsjln, SL, Wathen, NC, Tingulstad, S, Wiedswang, G, Sundan, A, Waage, A, Austgulen, R Tumor necrosis factor, interleukin-1, and interleukin-6 in normal human pregnancy. *Am J Obstet Gynecol* 1993; **169:** 397-404.

410. Ovaskainen, Y, Koponen, H, Jokelainen, J, Keinanen-Kiukaanniemi, S, Kumpusalo, E, Vanhala, M Depressive symptomatology is associated with decreased interleukin-1 beta and increased interleukin-1 receptor antagonist levels in males. *Psychiatry Res* 2009; **167:** 73-79.

411. Pagnin, D, de, Q, V, Pini, S, Cassano, GB Efficacy of ECT in depression: a meta-analytic review. *J ECT* 2004; **20:** 13-20.

412. Palsson, SP, Ostling, S, Skoog, I The incidence of first-onset depression in a population followed from the age of 70 to 85.

Psychol Med 2001; **31:** 1159-68.

413. Pankratov, YV, Lalo, UV, Krishtal, OA Role for P2X receptors in long-term potentiation. *J Neurosci* 2002; **22:** 8363-69.

414. Papakostas, GI Tolerability of modern antidepressants. *J Clin Psychiatry* 2008; **69 Suppl E1:** 8-13.

415. Papanicolaou, DA, Wilder, RL, Manolagas, SC, Chrousos, GP The pathophysiologic roles of interleukin-6 in human disease. *Ann Intern Med* 1998a; **128:** 127-37.

416. Papanicolaou, DA, Wilder, RL, Manolagas, SC, Chrousos, GP The pathophysiologic roles of interleukin-6 in human disease. *Ann Intern Med* 1998b; **128:** 127-37.

417. Pariante, CM The glucocorticoid receptor: part of the solution or part of the problem? *J Psychopharmacol* 2006; **20:** 79-84.

418. Pariante, CM, Lightman, SL The HPA axis in major depression: classical theories and new developments. *Trends Neurosci* 2008; **31:** 464-68.

419. Pariel-Madjlessi, S, Pouillon, M, Robcis, I, Sebban, C, Fremont, P, Belmin, J [Depression: an underrated consequence of stroke in elderly]. *Psychol Neuropsychiatr Vieil* 2005; **3:** 7-16.

420. Parvathenani, LK, Tertyshnikova, S, Greco, CR, Roberts, SB, Robertson, B, Posmantur, R P2X7 mediates superoxide production in primary microglia and is up-regulated in a transgenic mouse model of Alzheimer's disease. *J Biol Chem* 2003; **278:** 13309-17.

421. Patten, SB, Wang, JL, Williams, JV, Currie, S, Beck, CA, Maxwell, CJ, El-Guebaly, N Descriptive epidemiology of major depression in Canada. *Can J Psychiatry* 2006; **51:** 84-90.

422. Paykel, ES Life events and affective disorders. *Acta Psychiatr Scand Suppl* 2003; 61-66.

423. Payne, DK, Sullivan, MD, Massie, MJ Women's psychological reactions to breast cancer. *Semin Oncol* 1996; **23:** 89-97.

424. Pedersen, SH, Stage, KB, Bertelsen, A, Grinsted, P, Kragh-Sorensen, P, Sorensen, T ICD-10 criteria for depression in general practice. *J Affect Disord* 2001; **65:** 191-94.

425. Pellegatti, P, Raffaghello, L, Bianchi, G, Piccardi, F, Pistoia, V, Di, VF Increased level of extracellular ATP at tumor sites: in vivo imaging with plasma membrane luciferase. *PLoS One* 2008; **3:** e2599.

426. Penninx, BW, Beekman, AT, Honig, A, Deeg, DJ, Schoevers, RA, van Eijk, JT, van, TW Depression and cardiac mortality: results from a community-based longitudinal study. *Arch Gen Psychiatry* 2001;

58: 221-27.

427. Perregaux, D, Barberia, J, Lanzetti, AJ, Geoghegan, KF, Carty, TJ, Gabel, CA IL-1 beta maturation: evidence that mature cytokine formation can be induced specifically by nigericin. *J Immunol* 1992; **149:** 1294-303.

428. Perregaux, D, Gabel, CA Interleukin-1 beta maturation and release in response to ATP and nigericin. Evidence that potassium depletion mediated by these agents is a necessary and common feature of their activity. *J Biol Chem* 1994a; **269:** 15195-203.

429. Perregaux, D, Gabel, CA Interleukin-1 beta maturation and release in response to ATP and nigericin. Evidence that potassium depletion mediated by these agents is a necessary and common feature of their activity. *J Biol Chem* 1994b; **269:** 15195-203.

430. Perregaux, DG, Gabel, CA Post-translational processing of murine IL-1: evidence that ATP-induced release of IL-1 alpha and IL-1 beta occurs via a similar mechanism. *J Immunol* 1998; **160:** 2469-77.

431. Petrilli, V, Dostert, C, Muruve, DA, Tschopp, J The inflammasome: a danger sensing complex triggering innate immunity. *Curr Opin Immunol* 2007; **19:** 615-22.

432. Pincus, HA, Pettit, AR The societal costs of chronic major depression. *J Clin Psychiatry* 2001; **62 Suppl 6:** 5-9.

433. Plata-Salaman, CR Cytokine-induced anorexia. Behavioral, cellular, and molecular mechanisms. *Ann N Y Acad Sci* 1998; **856:** 160-170.

434. Plata-Salaman, CR, Oomura, Y, Kai, Y Tumor necrosis factor and interleukin-1 beta: suppression of food intake by direct action in the central nervous system. *Brain Res* 1988; **448:** 106-14.

435. Plotsky, PM, Owens, MJ, Nemeroff, CB Psychoneuroendocrinology of depression. Hypothalamic-pituitary-adrenal axis. *Psychiatr Clin North Am* 1998; **21:** 293-307.

436. Polazzi, E, Contestabile, A Reciprocal interactions between microglia and neurons: from survival to neuropathology. *Rev Neurosci* 2002; **13:** 221-42.

437. Pompili, M, Gibiino, S, Innamorati, M, Serafini, G, Del, CA, De, RL, Palermo, M, Montebovi, F, Campi, S, De, L, V, Sher, L, Tatarelli, R, Biondi, M, Duval, F, Serretti, A, Girardi, P Prolactin and thyroid hormone levels are associated with suicide attempts in psychiatric patients. *Psychiatry Res* 2012.

438. Prudic, J, Sackeim, HA Electroconvulsive therapy and suicide risk. *J Clin Psychiatry* 1999; **60 Suppl 2:** 104-10.

439. Pugh, CR, Nguyen, KT, Gonyea, JL, Fleshner, M, Wakins, LR, Maier, SF, Rudy, JW Role of interleukin-1 beta in impairment of contextual

fear conditioning caused by social isolation. *Behav Brain Res* 1999; **106:** 109-18.

440. Puthussery, T, Fletcher, EL Synaptic localization of P2X7 receptors in the rat retina. *J Comp Neurol* 2004; **472:** 13-23.

441. Qin, L, He, J, Hanes, RN, Pluzarev, O, Hong, JS, Crews, FT Increased systemic and brain cytokine production and neuroinflammation by endotoxin following ethanol treatment. *J Neuroinflammation* 2008; **5:** 10.

442. Qin, P, Agerbo, E, Westergard-Nielsen, N, Eriksson, T, Mortensen, PB Gender differences in risk factors for suicide in Denmark. *Br J Psychiatry* 2000; **177:** 546-50.

443. Qin, P, Nordentoft, M Suicide risk in relation to psychiatric hospitalization: evidence based on longitudinal registers. *Arch Gen Psychiatry* 2005a; **62:** 427-32.

444. Qin, P, Nordentoft, M Suicide risk in relation to psychiatric hospitalization: evidence based on longitudinal registers. *Arch Gen Psychiatry* 2005b; **62:** 427-32.

445. Raedler, TJ Inflammatory mechanisms in major depressive disorder. *Curr Opin Psychiatry* 2011; **24:** 519-25.

446. Raison, CL, Capuron, L, Miller, AH Cytokines sing the blues: inflammation and the pathogenesis of depression. *Trends Immunol* 2006; **27:** 24-31.

447. Raison, CL, Miller, AH Is depression an inflammatory disorder? *Curr Psychiatry Rep* 2011; **13:** 467-75.

448. Raju, TN The Nobel chronicles. 1927: Julius Wagner-Jauregg (1857-1940). *Lancet* 1998; **352:** 1714.

449. Raju, TN Hot brains: manipulating body heat to save the brain. *Pediatrics* 2006; **117:** e320-e321.

450. Rassendren, F, Buell, GN, Virginio, C, Collo, G, North, RA, Surprenant, A The permeabilizing ATP receptor, P2X7. Cloning and expression of a human cDNA. *J Biol Chem* 1997; **272:** 5482-86.

451. Ravichandran, KS Beginnings of a good apoptotic meal: the find-me and eat-me signaling pathways. *Immunity* 2011; **35:** 445-55.

452. Reddy, MS Depression: the disorder and the burden. *Indian J Psychol Med* 2010; **32:** 1-2.

453. Reddy, P Interleukin-18: recent advances. *Curr Opin Hematol* 2004; **11:** 405-10.

454. Reichenberg, A, Yirmiya, R, Schuld, A, Kraus, T, Haack, M, Morag, A, Pollmacher, T Cytokine-associated emotional and cognitive

disturbances in humans. *Arch Gen Psychiatry* 2001; **58:** 445-52.

455. Rethorst, CD, Toups, MS, Greer, TL, Nakonezny, PA, Carmody, TJ, Grannemann, BD, Huebinger, RM, Barber, RC, Trivedi, MH Pro-inflammatory cytokines as predictors of antidepressant effects of exercise in major depressive disorder. *Mol Psychiatry* 2012.

456. Rich, CL, Young, D, Fowler, RC San Diego suicide study. I. Young vs old subjects. *Arch Gen Psychiatry* 1986; **43:** 577-82.

457. Rihmer, Z, Gonda, X Prevention of depression-related suicides in primary care. *Psychiatr Hung* 2012; **27:** 72-81.

458. Roger, S, Mei, ZZ, Baldwin, JM, Dong, L, Bradley, H, Baldwin, SA, Surprenant, A, Jiang, LH Single nucleotide polymorphisms that were identified in affective mood disorders affect ATP-activated P2X7 receptor functions. *J Psychiatr Res* 2010; **44:** 347-55.

459. Romera, I, Perez, V, Manuel, MJ, Schacht, A, Papen, R, Neuhauser, D, Abbar, M, Picard, H, Gilaberte, I Early vs. conventional switching of antidepressants in patients with MDD and moderate to severe pain: A double-blind randomized study. *J Affect Disord* 2012.

460. Ross, GD Regulation of the adhesion versus cytotoxic functions of the Mac-1/CR3/alphaMbeta2-integrin glycoprotein. *Crit Rev Immunol* 2000; **20:** 197-222.

461. Roy, A, Nielsen, D, Rylander, G, Sarchiapone, M, Segal, N Genetics of suicide in depression. *J Clin Psychiatry* 1999; **60 Suppl 2:** 12-17.

462. Rubin, RT, Poland, RE, Lesser, IM, Winston, RA, Blodgett, AL Neuroendocrine aspects of primary endogenous depression. I. Cortisol secretory dynamics in patients and matched controls. *Arch Gen Psychiatry* 1987; **44:** 328-36.

463. Saarelainen, T, Hendolin, P, Lucas, G, Koponen, E, Sairanen, M, MacDonald, E, Agerman, K, Haapasalo, A, Nawa, H, Aloyz, R, Ernfors, P, Castren, E Activation of the TrkB neurotrophin receptor is induced by antidepressant drugs and is required for antidepressant-induced behavioral effects. *J Neurosci* 2003; **23:** 349-57.

464. Sabljic, V, Ruzic, K, Rakun, R Venlafaxine withdrawal syndrome. *Psychiatr Danub* 2011; **23:** 117-19.

465. Sackeim, HA The definition and meaning of treatment-resistant depression. *J Clin Psychiatry* 2001; **62 Suppl 16:** 10-17.

466. Sairanen, M, Lucas, G, Ernfors, P, Castren, M, Castren, E Brain-derived neurotrophic factor and antidepressant drugs have different but coordinated effects on neuronal turnover, proliferation, and survival in the adult dentate gyrus. *J Neurosci* 2005; **25:** 1089-94.

467. Salter, MW, Hicks, JL ATP-evoked increases in intracellular calcium in neurons and glia from the dorsal spinal cord. *J Neurosci* 1994; **14:** 1563-75.

468. Sanchez, MM, Ladd, CO, Plotsky, PM Early adverse experience as a developmental risk factor for later psychopathology: evidence from rodent and primate models. *Dev Psychopathol* 2001; **13:** 419-49.

469. Sandeep Grover, Surendra Kumar Mattoo, and Nitin Gupta. Theories on Mechanism of Action of Electroconvulsive Therapy. 8(4), 70-84. 2005.
Ref Type: Generic

470. Sansone, RA, Sansone, LA Agomelatine: A Novel Antidepressant. *Innov Clin Neurosci* 2011; **8:** 10-14.

471. Santarelli, L, Saxe, M, Gross, C, Surget, A, Battaglia, F, Dulawa, S, Weisstaub, N, Lee, J, Duman, R, Arancio, O, Belzung, C, Hen, R Requirement of hippocampal neurogenesis for the behavioral effects of antidepressants. *Science* 2003; **301:** 805-9.

472. Sanz, JM, Di, VF Kinetics and mechanism of ATP-dependent IL-1 beta release from microglial cells. *J Immunol* 2000; **164:** 4893-98.

473. Sapolsky, R, Rivier, C, Yamamoto, G, Plotsky, P, Vale, W Interleukin-1 stimulates the secretion of hypothalamic corticotropin-releasing factor. *Science* 1987a; **238:** 522-24.

474. Sapolsky, R, Rivier, C, Yamamoto, G, Plotsky, P, Vale, W Interleukin-1 stimulates the secretion of hypothalamic corticotropin-releasing factor. *Science* 1987b; **238:** 522-24.

475. Schaefer, M, Schwaiger, M, Garkisch, AS, Pich, M, Hinzpeter, A, Uebelhack, R, Heinz, A, van, BF, Berg, T Prevention of interferon-alpha associated depression in psychiatric risk patients with chronic hepatitis C. *J Hepatol* 2005; **42:** 793-98.

476. Scheibel, RS, Valentine, AD, O'Brien, S, Meyers, CA Cognitive dysfunction and depression during treatment with interferon-alpha and chemotherapy. *J Neuropsychiatry Clin Neurosci* 2004; **16:** 185-91.

477. Schiepers, OJ, Wichers, MC, Maes, M Cytokines and major depression. *Prog Neuropsychopharmacol Biol Psychiatry* 2005a; **29:** 201-17.

478. Schiepers, OJ, Wichers, MC, Maes, M Cytokines and major depression. *Prog Neuropsychopharmacol Biol Psychiatry* 2005b; **29:** 201-17.

479. Schildkraut, JJ, Kety, SS Biogenic amines and emotion. *Science* 1967; **156:** 21-37.

480. Schilling, WP, Wasylyna, T, Dubyak, GR, Humphreys, BD, Sinkins,

WG Maitotoxin and P2Z/P2X(7) purinergic receptor stimulation activate a common cytolytic pore. *Am J Physiol* 1999; **277:** C766-C776.

481. Schrocksnadel, K, Wirleitner, B, Winkler, C, Fuchs, D Monitoring tryptophan metabolism in chronic immune activation. *Clin Chim Acta* 2006; **364:** 82-90.

482. Schwarcz, R, Whetsell, WO, Jr., Mangano, RM Quinolinic acid: an endogenous metabolite that produces axon-sparing lesions in rat brain. *Science* 1983; **219:** 316-18.

483. Seckl, JR, Meaney, MJ Glucocorticoid programming. *Ann N Y Acad Sci* 2004; **1032:** 63-84.

484. Sheline, YI, Barch, DM, Donnelly, JM, Ollinger, JM, Snyder, AZ, Mintun, MA Increased amygdala response to masked emotional faces in depressed subjects resolves with antidepressant treatment: an fMRI study. *Biol Psychiatry* 2001; **50:** 651-58.

485. Shen, N, Tsao, BP Current advances in the human lupus genetics. *Curr Rheumatol Rep* 2004; **6:** 391-98.

486. Sherwood, A, Hinderliter, AL, Watkins, LL, Waugh, RA, Blumenthal, JA Impaired endothelial function in coronary heart disease patients with depressive symptomatology. *J Am Coll Cardiol* 2005; **46:** 656-59.

487. Shields, LB, Hunsaker, DM, Hunsaker, JC, III Suicide: a ten-year retrospective review of Kentucky medical examiner cases. *J Forensic Sci* 2005; **50:** 613-17.

488. Shigetomi, E, Kato, F Action potential-independent release of glutamate by Ca2+ entry through presynaptic P2X receptors elicits postsynaptic firing in the brainstem autonomic network. *J Neurosci* 2004; **24:** 3125-35.

489. Shin, DI, Banning, U, Kim, YM, Verheyen, J, Hannen, M, Bonig, H, Korholz, D Interleukin 10 inhibits TNF-alpha production in human monocytes independently of interleukin 12 and interleukin 1 beta. *Immunol Invest* 1999; **28:** 165-75.

490. Shintani, F, Nakaki, T, Kanba, S, Sato, K, Yagi, G, Shiozawa, M, Aiso, S, Kato, R, Asai, M Involvement of interleukin-1 in immobilization stress-induced increase in plasma adrenocorticotropic hormone and in release of hypothalamic monoamines in the rat. *J Neurosci* 1995; **15:** 1961-70.

491. Siegle, GJ, Steinhauer, SR, Thase, ME, Stenger, VA, Carter, CS Can't shake that feeling: event-related fMRI assessment of sustained amygdala activity in response to emotional information in depressed individuals. *Biol Psychiatry* 2002; **51:** 693-707.

492. Silberg, J, Pickles, A, Rutter, M, Hewitt, J, Simonoff, E, Maes, H,

Carbonneau, R, Murrelle, L, Foley, D, Eaves, L The influence of genetic factors and life stress on depression among adolescent girls. *Arch Gen Psychiatry* 1999a; **56:** 225-32.

493. Silberg, J, Pickles, A, Rutter, M, Hewitt, J, Simonoff, E, Maes, H, Carbonneau, R, Murrelle, L, Foley, D, Eaves, L The influence of genetic factors and life stress on depression among adolescent girls. *Arch Gen Psychiatry* 1999b; **56:** 225-32.

494. Simen, BB, Duman, CH, Simen, AA, Duman, RS TNFalpha signaling in depression and anxiety: behavioral consequences of individual receptor targeting. *Biol Psychiatry* 2006; **59:** 775-85.

495. Slattery, DA, Hudson, AL, Nutt, DJ Invited review: the evolution of antidepressant mechanisms. *Fundam Clin Pharmacol* 2004; **18:** 1-21.

496. Sluyter, R, Shemon, AN, Wiley, JS Glu496 to Ala polymorphism in the P2X7 receptor impairs ATP-induced IL-1 beta release from human monocytes. *J Immunol* 2004; **172:** 3399-405.

497. Sluzewska, A, Rybakowski, JK, Laciak, M, Mackiewicz, A, Sobieska, M, Wiktorowicz, K Interleukin-6 serum levels in depressed patients before and after treatment with fluoxetine. *Ann N Y Acad Sci* 1995; **762:** 474-76.

498. Smith, EM, Cadet, P, Stefano, GB, Opp, MR, Hughes, TK, Jr. IL-10 as a mediator in the HPA axis and brain. *J Neuroimmunol* 1999; **100:** 140-148.

499. Smith, KA, Fairburn, CG, Cowen, PJ Relapse of depression after rapid depletion of tryptophan. *Lancet* 1997; **349:** 915-19.

500. Smith, RS The macrophage theory of depression. *Med Hypotheses* 1991a; **35:** 298-306.

501. Smith, RS The macrophage theory of depression. *Med Hypotheses* 1991b; **35:** 298-306.

502. Solle, M, Labasi, J, Perregaux, DG, Stam, E, Petrushova, N, Koller, BH, Griffiths, RJ, Gabel, CA Altered cytokine production in mice lacking P2X(7) receptors. *J Biol Chem* 2001; **276:** 125-32.

503. Song, C, Dinan, T, Leonard, BE Changes in immunoglobulin, complement and acute phase protein levels in the depressed patients and normal controls. *J Affect Disord* 1994; **30:** 283-88.

504. Song, C, Wang, H Cytokines mediated inflammation and decreased neurogenesis in animal models of depression. *Prog Neuropsychopharmacol Biol Psychiatry* 2011; **35:** 760-768.

505. Sonino, N, Fava, GA, Raffi, AR, Boscaro, M, Fallo, F Clinical correlates of major depression in Cushing's disease. *Psychopathology* 1998;

31: 302-6.

506. Spalletta, G, Bossu, P, Ciaramella, A, Bria, P, Caltagirone, C, Robinson, RG The etiology of poststroke depression: a review of the literature and a new hypothesis involving inflammatory cytokines. *Mol Psychiatry* 2006; **11:** 984-91.

507. Spera, PA, Ellison, JA, Feuerstein, GZ, Barone, FC IL-10 reduces rat brain injury following focal stroke. *Neurosci Lett* 1998; **251:** 189-92.

508. Sperlagh, B, Heinrich, A, Csolle, C P2 receptor-mediated modulation of neurotransmitter release-an update. *Purinergic Signal* 2007; **3:** 269-84.

509. Sperlagh, B, Vizi, ES, Wirkner, K, Illes, P P2X7 receptors in the nervous system. *Prog Neurobiol* 2006; **78:** 327-46.

510. Sperner-Unterweger, B, Barnas, C, Fuchs, D, Kemmler, G, Wachter, H, Hinterhuber, H, Fleischhacker, WW Neopterin production in acute schizophrenic patients: an indicator of alterations of cell-mediated immunity. *Psychiatry Res* 1992; **42:** 121-28.

511. Spulber, S, Oprica, M, Bartfai, T, Winblad, B, Schultzberg, M Blunted neurogenesis and gliosis due to transgenic overexpression of human soluble IL-1ra in the mouse. *Eur J Neurosci* 2008; **27:** 549-58.

512. Steinberg, TH, Newman, AS, Swanson, JA, Silverstein, SC *J Biol Chem* 1987a; **262:** 8884-88.

513. Steinberg, TH, Newman, AS, Swanson, JA, Silverstein, SC ATP4- permeabilizes the plasma membrane of mouse macrophages to fluorescent dyes. *J Biol Chem* 1987b; **262:** 8884-88.

514. Steinberg, TH, Silverstein, SC Extracellular ATP4- promotes cation fluxes in the J774 mouse macrophage cell line. *J Biol Chem* 1987a; **262:** 3118-22.

515. Steinberg, TH, Silverstein, SC Extracellular ATP4- promotes cation fluxes in the J774 mouse macrophage cell line. *J Biol Chem* 1987b; **262:** 3118-22.

516. Stock, C, Schilling, T, Schwab, A, Eder, C Lysophosphatidylcholine stimulates IL-1beta release from microglia via a P2X7 receptor-independent mechanism. *J Immunol* 2006; **177:** 8560-8568.

517. Stokes, L, Fuller, SJ, Sluyter, R, Skarratt, KK, Gu, BJ, Wiley, JS Two haplotypes of the P2X(7) receptor containing the Ala-348 to Thr polymorphism exhibit a gain-of-function effect and enhanced interleukin-1beta secretion. *FASEB J* 2010; **24:** 2916-27.

518. Stokes, PE The potential role of excessive cortisol induced by HPA hyperfunction in the pathogenesis of depression. *Eur*

Neuropsychopharmacol 1995; **5 Suppl:** 77-82.

519. Stommel, M, Given, BA, Given, CW Depression and functional status as predictors of death among cancer patients. *Cancer* 2002; **94:** 2719-27.

520. Stone, TW, Behan, WM Interleukin-1beta but not tumor necrosis factor-alpha potentiates neuronal damage by quinolinic acid: protection by an adenosine A2A receptor antagonist. *J Neurosci Res* 2007; **85:** 1077-85.

521. Stone, TW, Perkins, MN Quinolinic acid: a potent endogenous excitant at amino acid receptors in CNS. *Eur J Pharmacol* 1981; **72:** 411-12.

522. Streit, WJ Microglia and Alzheimer's disease pathogenesis. *J Neurosci Res* 2004; **77:** 1-8.

523. Surget, A, Tanti, A, Leonardo, ED, Laugeray, A, Rainer, Q, Touma, C, Palme, R, Griebel, G, Ibarguen-Vargas, Y, Hen, R, Belzung, C Antidepressants recruit new neurons to improve stress response regulation. *Mol Psychiatry* 2011a; **16:** 1177-88.

524. Surget, A, Tanti, A, Leonardo, ED, Laugeray, A, Rainer, Q, Touma, C, Palme, R, Griebel, G, Ibarguen-Vargas, Y, Hen, R, Belzung, C Antidepressants recruit new neurons to improve stress response regulation. *Mol Psychiatry* 2011b; **16:** 1177-88.

525. Surprenant, A, Rassendren, F, Kawashima, E, North, RA, Buell, G The cytolytic P2Z receptor for extracellular ATP identified as a P2X receptor (P2X7). *Science* 1996; **272:** 735-38.

526. Sutton, RE, Koob, GF, Le, MM, Rivier, J, Vale, W Corticotropin releasing factor produces behavioural activation in rats. *Nature* 1982; **297:** 331-33.

527. Szczepanska-Sadowska, E, Cudnoch-Jedrzejewska, A, Ufnal, M, Zera, T Brain and cardiovascular diseases: common neurogenic background of cardiovascular, metabolic and inflammatory diseases. *J Physiol Pharmacol* 2010; **61:** 509-21.

528. Teicher, MH, Anderson, CM, Polcari, A Childhood maltreatment is associated with reduced volume in the hippocampal subfields CA3, dentate gyrus, and subiculum. *Proc Natl Acad Sci U S A* 2012; **109:** E563-E572.

529. Thase, ME, Entsuah, AR, Rudolph, RL Remission rates during treatment with venlafaxine or selective serotonin reuptake inhibitors. *Br J Psychiatry* 2001; **178:** 234-41.

530. Thase, ME, Haight, BR, Richard, N, Rockett, CB, Mitton, M, Modell, JG, VanMeter, S, Harriett, AE, Wang, Y Remission rates following antidepressant therapy with bupropion or selective serotonin reuptake inhibitors: a meta-analysis of original data from 7

randomized controlled trials. *J Clin Psychiatry* 2005; **66:** 974-81.

531. Thayer, JF, Sternberg, EM Neural aspects of immunomodulation: focus on the vagus nerve. *Brain Behav Immun* 2010; **24:** 1223-28.

532. Thomas, AJ, Davis, S, Morris, C, Jackson, E, Harrison, R, O'Brien, JT Increase in interleukin-1beta in late-life depression. *Am J Psychiatry* 2005; **162:** 175-77.

533. Thuile, J, Even, C, Guelfi, JD [Validity of vascular depression as a specific diagnostic: a review]. *Encephale* 2007; **33:** 39-48.

534. Tomfohr, LM Multiwave associations between depressive symptoms and endothelial function in adolescent and young adult females. 2011.

535. Tomfohr, LM, Martin, TM, Miller, GE Symptoms of depression and impaired endothelial function in healthy adolescent women. *J Behav Med* 2008; **31:** 137-43.

536. Trask, PC, Esper, P, Riba, M, Redman, B Psychiatric side effects of interferon therapy: prevalence, proposed mechanisms, and future directions. *J Clin Oncol* 2000; **18:** 2316-26.

537. Tu, H, Rady, PL, Juelich, T, Smith, EM, Tyring, SK, Hughes, TK Cytokine regulation of tryptophan metabolism in the hypothalamic-pituitary-adrenal (HPA) axis: implications for protective and toxic consequences in neuroendocrine regulation. *Cell Mol Neurobiol* 2005; **25:** 673-80.

538. Tuglu, C, Kara, SH, Caliyurt, O, Vardar, E, Abay, E Increased serum tumor necrosis factor-alpha levels and treatment response in major depressive disorder. *Psychopharmacology (Berl)* 2003; **170:** 429-33.

539. Tundo, A, Proietti, L, Cavalieri, P [Pharmacotherapy plus psychotherapy in patients with mood disorder and Axis II codiagnosis. A review]. *Riv Psichiatr* 2012; **47:** 226-30.

540. Tyring, S, Gottlieb, A, Papp, K, Gordon, K, Leonardi, C, Wang, A, Lalla, D, Woolley, M, Jahreis, A, Zitnik, R, Cella, D, Krishnan, R Etanercept and clinical outcomes, fatigue, and depression in psoriasis: double-blind placebo-controlled randomised phase III trial. *Lancet* 2006; **367:** 29-35.

541. van Dam, AM, Brouns, M, Louisse, S, Berkenbosch, F Appearance of interleukin-1 in macrophages and in ramified microglia in the brain of endotoxin-treated rats: a pathway for the induction of non-specific symptoms of sickness? *Brain Res* 1992; **588:** 291-96.

542. Visentin, S, Renzi, M, Frank, C, Greco, A, Levi, G Two different ionotropic receptors are activated by ATP in rat microglia. *J Physiol* 1999; **519 Pt 3:** 723-36.

543. Vitkovic, L, Bockaert, J, Jacque, C "Inflammatory" cytokines: neuromodulators in normal brain? *J Neurochem* 2000; **74:** 457-71.

544. Voderholzer, U Sleep deprivation and antidepressant treatment. *Dialogues Clin Neurosci* 2003; **5:** 366-69.

545. Vukovic, O, Maric, NP, Britvic, D, Cvetic, T, Damjanovic, A, Prostran, M, Jasovic-Gasic, M Efficacy, tolerability and safety of tianeptine in special populations of depressive patients. *Psychiatr Danub* 2009; **21:** 194-98.

546. Vythilingam, M, Heim, C, Newport, J, Miller, AH, Anderson, E, Bronen, R, Brummer, M, Staib, L, Vermetten, E, Charney, DS, Nemeroff, CB, Bremner, JD Childhood trauma associated with smaller hippocampal volume in women with major depression. *Am J Psychiatry* 2002; **159:** 2072-80.

547. Wagner, J, Tennen, H, Mansoor, G, Abbott, G Endothelial dysfunction and history of recurrent depression in postmenopausal women with Type 2 diabetes: a case-control study. *J Diabetes Complications* 2009; **23:** 18-24.

548. Wagner-Jauregg, J, BRUETSCH, WL The history of the malaria treatment of general paralysis. *Am J Psychiatry* 1946; **102:** 577-82.

549. Wake, H, Moorhouse, AJ, Jinno, S, Kohsaka, S, Nabekura, J Resting microglia directly monitor the functional state of synapses in vivo and determine the fate of ischemic terminals. *J Neurosci* 2009; **29:** 3974-80.

550. Wang, X, Arcuino, G, Takano, T, Lin, J, Peng, WG, Wan, P, Li, P, Xu, Q, Liu, QS, Goldman, SA, Nedergaard, M P2X7 receptor inhibition improves recovery after spinal cord injury. *Nat Med* 2004; **10:** 821-27.

551. Watkins, LR, Maier, SF, Goehler, LE Cytokine-to-brain communication: a review & analysis of alternative mechanisms. *Life Sci* 1995; **57:** 1011-26.

552. Watson, M, Greer, S, Blake, S, Shrapnell, K Reaction to a diagnosis of breast cancer. Relationship between denial, delay and rates of psychological morbidity. *Cancer* 1984; **53:** 2008-12.

553. Weissman, MM, Wolk, S, Goldstein, RB, Moreau, D, Adams, P, Greenwald, S, Klier, CM, Ryan, ND, Dahl, RE, Wickramaratne, P Depressed adolescents grown up. *JAMA* 1999; **281:** 1707-13.

554. Werry, EL, Liu, GJ, Bennett, MR Glutamate-stimulated ATP release from spinal cord astrocytes is potentiated by substance P. *J Neurochem* 2006; **99:** 924-36.

555. Whyte, EM, Mulsant, BH Post stroke depression: epidemiology, pathophysiology, and biological treatment. *Biol Psychiatry* 2002;

52: 253-64.

556. Wichers, MC, Koek, GH, Robaeys, G, Verkerk, R, Scharpe, S, Maes, M IDO and interferon-alpha-induced depressive symptoms: a shift in hypothesis from tryptophan depletion to neurotoxicity. *Mol Psychiatry* 2005; **10:** 538-44.

557. Wichers, MC, Maes, M The role of indoleamine 2,3-dioxygenase (IDO) in the pathophysiology of interferon-alpha-induced depression. *J Psychiatry Neurosci* 2004; **29:** 11-17.

558. Wiley, JS, Dao-Ung, LP, Li, C, Shemon, AN, Gu, BJ, Smart, ML, Fuller, SJ, Barden, JA, Petrou, S, Sluyter, R An Ile-568 to Asn polymorphism prevents normal trafficking and function of the human P2X7 receptor. *J Biol Chem* 2003; **278:** 17108-13.

559. Williams, SA, Kasl, SV, Heiat, A, Abramson, JL, Krumholz, HM, Vaccarino, V Depression and risk of heart failure among the elderly: a prospective community-based study. *Psychosom Med* 2002; **64:** 6-12.

560. Willner, P The validity of animal models of depression. *Psychopharmacology (Berl)* 1984a; **83:** 1-16.

561. Willner, P The validity of animal models of depression. *Psychopharmacology (Berl)* 1984b; **83:** 1-16.

562. Willner, P Validation criteria for animal models of human mental disorders: learned helplessness as a paradigm case. *Prog Neuropsychopharmacol Biol Psychiatry* 1986; **10:** 677-90.

563. Willner, P Validity, reliability and utility of the chronic mild stress model of depression: a 10-year review and evaluation. *Psychopharmacology (Berl)* 1997; **134:** 319-29.

564. Willner, P, Muscat, R, Papp, M Chronic mild stress-induced anhedonia: a realistic animal model of depression. *Neurosci Biobehav Rev* 1992; **16:** 525-34.

565. Wilson, CJ, Finch, CE, Cohen, HJ Cytokines and cognition--the case for a head-to-toe inflammatory paradigm. *J Am Geriatr Soc* 2002; **50:** 2041-56.

566. Wimbiscus, M, Kostenko, O, Malone, D MAO inhibitors: risks, benefits, and lore. *Cleve Clin J Med* 2010; **77:** 859-82.

567. Wittchen, HU, Jacobi, F, Rehm, J, Gustavsson, A, Svensson, M, Jonsson, B, Olesen, J, Allgulander, C, Alonso, J, Faravelli, C, Fratiglioni, L, Jennum, P, Lieb, R, Maercker, A, van, OJ, Preisig, M, Salvador-Carulla, L, Simon, R, Steinhausen, HC The size and burden of mental disorders and other disorders of the brain in Europe 2010. *Eur Neuropsychopharmacol* 2011; **21:** 655-79.

568. Wong, EY, Herbert, J The corticoid environment: a determining

factor for neural progenitors' survival in the adult hippocampus. *Eur J Neurosci* 2004; **20:** 2491-98.

569. Wong, EY, Herbert, J Raised circulating corticosterone inhibits neuronal differentiation of progenitor cells in the adult hippocampus. *Neuroscience* 2006; **137:** 83-92.

570. Wong, ML, Licinio, J From monoamines to genomic targets: a paradigm shift for drug discovery in depression. *Nat Rev Drug Discov* 2004; **3:** 136-51.

571. Wood, K, Harwood, J, Coppen, A The effect of antidepressant drugs on plasma kynurenine in depressed patients. *Psychopharmacology (Berl)* 1978; **59:** 263-66.

572. Wu, MD, Hein, AM, Moravan, MJ, Shaftel, SS, Olschowka, JA, O'Banion, MK Adult murine hippocampal neurogenesis is inhibited by sustained IL-1beta and not rescued by voluntary running. *Brain Behav Immun* 2012; **26:** 292-300.

573. Wu, ML, Deng, JF Fatal serotonin toxicity caused by moclobemide and fluoxetine overdose. *Chang Gung Med J* 2011; **34:** 644-49.

574. Wu, TT, Chen, TL, Chen, RM Lipopolysaccharide triggers macrophage activation of inflammatory cytokine expression, chemotaxis, phagocytosis, and oxidative ability via a toll-like receptor 4-dependent pathway: validated by RNA interference. *Toxicol Lett* 2009; **191:** 195-202.

575. Wulsin, LR, Singal, BM Do depressive symptoms increase the risk for the onset of coronary disease? A systematic quantitative review. *Psychosom Med* 2003; **65:** 201-10.

576. Xia, Z, DePierre, JW, Nassberger, L Tricyclic antidepressants inhibit IL-6, IL-1 beta and TNF-alpha release in human blood monocytes and IL-2 and interferon-gamma in T cells. *Immunopharmacology* 1996; **34:** 27-37.

577. Xu, Y, Sari, Y, Zhou, FC Selective serotonin reuptake inhibitor disrupts organization of thalamocortical somatosensory barrels during development. *Brain Res Dev Brain Res* 2004; **150:** 151-61.

578. Yalcin, I, Belzung, C, Surget, A Mouse strain differences in the unpredictable chronic mild stress: a four-antidepressant survey. *Behav Brain Res* 2008; **193:** 140-143.

579. Yao, J, Keri, JE, Taffs, RE, Colton, CA Characterization of interleukin-1 production by microglia in culture. *Brain Res* 1992; **591:** 88-93.

580. Yates, WR, Gleason, O Hepatitis C and depression. *Depress Anxiety* 1998; **7:** 188-93.

581. Yirmiya, R Depression in medical illness: the role of the immune

system. *West J Med* 2000; **173:** 333-36.

582. Yirmiya, R, Pollak, Y, Morag, M, Reichenberg, A, Barak, O, Avitsur, R, Shavit, Y, Ovadia, H, Weidenfeld, J, Morag, A, Newman, ME, Pollmacher, T Illness, cytokines, and depression. *Ann N Y Acad Sci* 2000a; **917:** 478-87.

583. Yirmiya, R, Pollak, Y, Morag, M, Reichenberg, A, Barak, O, Avitsur, R, Shavit, Y, Ovadia, H, Weidenfeld, J, Morag, A, Newman, ME, Pollmacher, T Illness, cytokines, and depression. *Ann N Y Acad Sci* 2000b; **917:** 478-87.

584. Yoshihisa Kitamura, Daijiro Yanagisawa, Kazuyuki Takata, Takashi Taniguchi Neuroprotective function in brain microglia. *Current Anaesthesia & Critical Care* 2009; **20:** 142-47.

585. Yudkin, JS, Kumari, M, Humphries, SE, Mohamed-Ali, V Inflammation, obesity, stress and coronary heart disease: is interleukin-6 the link? *Atherosclerosis* 2000a; **148:** 209-14.

586. Yudkin, JS, Kumari, M, Humphries, SE, Mohamed-Ali, V Inflammation, obesity, stress and coronary heart disease: is interleukin-6 the link? *Atherosclerosis* 2000b; **148:** 209-14.

587. Yudkin, JS, Yajnik, CS, Mohamed-Ali, V, Bulmer, K High levels of circulating proinflammatory cytokines and leptin in urban, but not rural, Indians. A potential explanation for increased risk of diabetes and coronary heart disease. *Diabetes Care* 1999; **22:** 363-64.

588. Zainab, S, Fatmi, Z, Kazi, A Risk factors for depression among married women belonging to higher and lower socioeconomic status in Karachi, Pakistan. *J Pak Med Assoc* 2012; **62:** 249-53.

589. Zarate, CA, Jr., Singh, JB, Carlson, PJ, Brutsche, NE, Ameli, R, Luckenbaugh, DA, Charney, DS, Manji, HK A randomized trial of an N-methyl-D-aspartate antagonist in treatment-resistant major depression. *Arch Gen Psychiatry* 2006; **63:** 856-64.

590. Zhang, T, Chen, Y, Liu, H, Zhou, Z, Zhai, Y, Yang, J Chronic unpredictable stress accelerates atherosclerosis through promoting inflammation in apolipoprotein E knockout mice. *Thromb Res* 2010.

591. Zhu, B, Dong, Y, Xu, Z, Gompf, HS, Ward, SA, Xue, Z, Miao, C, Zhang, Y, Chamberlin, NL, Xie, Z Sleep disturbance induces neuroinflammation and impairment of learning and memory. *Neurobiol Dis* 2012.

592. Zunszain, PA, Anacker, C, Cattaneo, A, Carvalho, LA, Pariante, CM Glucocorticoids, cytokines and brain abnormalities in depression. *Prog Neuropsychopharmacol Biol Psychiatry* 2011; **35:** 722-29.

593. Zunszain, PA, Anacker, C, Cattaneo, A, Choudhury, S, Musaelyan, K, Myint, AM, Thuret, S, Price, J, Pariante, CM Interleukin-1beta: a new regulator of the kynurenine pathway affecting human hippocampal

neurogenesis. *Neuropsychopharmacology* 2012; **37:** 939-49.

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Functional implication of P2X7 receptor in neuroinflammatory phenomena associated with depression: A preclinical study

Major depression is a serious public health problem considering its association to a high risk of death by suicide, to major costs of treatment as well as a high rate of induced disability. It is prevalent throughout the developed and developing world. Major depression is characterized by symptoms such as low mood, feeling of helplessness and worthlessness, disturbances of bodily functions (sleep, appetite and libido), psychomotor retardation and suicidal ideation and behaviors. Last two decades has also seen an accumulation of evidence regarding the pathophysiology of depression, particularly suggesting an implication of various neurotransmitters systems such as noradrenaline, serotonin, as well as neuroendocrine modifications, in particular a hyperactivation of the HPA axis. However, the clinical evidence of an existing over-expression of inflammatory parameters in depressed subjects as well as increased prevalence of depressive symptoms in patients suffering from chronic inflammatory conditions or treated by cytokine chemotherapy, has added yet another pathophysiological potential mechanism in field of research. Among the pro-inflammatory cytokines that participate in the process of inflammation, IL-1β has a significant role. Release and maturation of IL-1β is driven in part by P2X7 receptors, a ligand gated purinergic receptor. Interestingly, polymorphism of the gene coding for P2X7 has been shown to be associated with increase vulnerability to mood disorder and neurodegenerative disorders.

The research project presented in this thesis aims at characterizing the inflammatory component (and particularly the role of neuroinflammation) of depression and the role of P2X7 receptor in this phenomena.. In a first experiment, we show that mice submitted to the Unpredictable Chronic Mild Stress (UCMS) protocol (but not mice in non-stressed condition) present significant microglial activation in stress responsive regions of brain but no alteration in the concentration of circulating cytokines. In a second experiment we aimed at comparing the effect of SSRI (fluoxetine) and a P2X7 receptor antagonism (BBG) on the UCMS induced behavioral, neuroendocrine and inflammatory and brain immunohistochemistry changes. Our results show that both fluoxetine and BBG reverse behavioral and HPA axis changes in mice submitted to UCMS. However Fluoxetine but not BBG increases the density of double-cortin positive cells (reflecting hippocampal neurogenesis) in the dentate gyrus of stressed animals

These results demonstrate that there is a neuroinflammatory component of depressive illness and highlight the fact that P2X7 receptor antagonist BBG (albeit non-specific) has similar effect on depression like behavior and on HPA axis changes as that of Fluoxetine, but no effect on neurogenesis. These results encourage potential of basic research on the role of neuroinflammation and particularly IL1-b and P2X7 as a component of depressive illness, emphasizing the antidepressant role of P2X7 receptors antagonists as a potential new class of antidepressants.

Key words: Neuroinflammation, Major depression, UCMS, P2X7 receptor, neurogenesis, HPA-axis, neurogenesis, Antidepressant resistance, IL-1β, microglia